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An extensive analysis of organics and inorganics present in leachate from landfills located in different regions of the United States was performed to evaluate methods for treating leachates. Bench-scale performance data and evaluation of leachate treatment methods are reported in Volume II. Gross properties of the leachate quality varied greatly between different landfills. Membrane ultrafiltration, gelpermeation chromatography, and specific organic analysis were used to separate different molecular weight fractions and to determine the main classes of organics and associated functional groups. The majority of the organics were able to permeate a 500 MW UF membrane indicative of their low molecular weight. Membrane fractionation and organic analysis of leachate samples collected from different landfills generally showed a decrease of the free volatile fatty acid fraction with increasing age of the fill. Biological degradation studies showed the sequential removal of different classes of organics. Four sequential phases were recognized: 1) removal of high molecular weight humic carbohydrate-like organics; 2) removal of free volatile fatty acids; 3) removal of bacterially excreted carbonyl compounds and amino acids; 4) removal of high molecular weight carbohydrates produced during the third phase. Membrane fractionation analysis showed that the majority of the metals permeated the 500 MW UF membrane, indicating that chelation of most metals by refractory organics in leachate plays a minor role in metal attenuation processes; an exception was iron, most of which was associated with the 100,000 MW UF retentate.

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Volume I

Characterization of Leachate

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from municipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

This study involved extensive analysis of different organics and inorganics present in leachate samples from landfills located in different regions of the United States. These analyses were then used to predict the effectiveness of different biological and physical-chemical treatment methods for contaminant removal. Laboratory bench-scale studies were then performed to determine the treatability of leachates of different compositions.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

The present study involved an extensive analysis of both organics and inorganics present in leachate samples collected from landfills located in different regions of the United States. Bench-scale performance data for selected leachate treatment methods were obtained. The character of the analyzed leachate samples was described using ratios of different parameters such as COD/TOC, BOD_5/COD, VS/FS and (total organic carbons present in the free volatile fatty acids)/TOC. Leachate from recently constructed landfills, characterized by high values for the listed ratios, was best treated by aerobic or anaerobic, biological treatment processes. Physical-chemical treatment processes, especially activated carbon and reverse osmosis, were found most effective in treating leachate from stabilized old landfills and were also useful for removing organics in effluent of biological units.

The leachate samples were subjected to membrane ultrafiltration, gelpermeation chromatography and specific organic analysis to separate different molecular weight fractions and to determine the main classes of organics and associated functional groups. The majority of the organics were able to permeate a 500 MW UF membrane, indicative of their low molecular weight. Most of the organics in the 500 MW UF permeate were free volatile fatty acids. Fulvic-like material with a relatively high carboxyl and aromatic hydroxyl group density was found to be the next largest group of organics. Membrane fractionation and organic analysis of leachate samples collected from different landfills commonly showed a decrease in the free volatile fatty acid fraction with increasing age of the fill.

Biological degradation studies showed the sequential removal of different classes of organics present in leachate from a recently constructed solid waste lysimeter. Four sequential phases were recognized: 1) removal of high molecular weight humic carbohydrate-like organics, 2) removal of free volatile fatty acids, 3) removal of bacterially excreted carbonyl compounds and amino acids, and 4) removal of high molecular weight carbohydrates produced during the third phase. A decreased ability of bacteria to degrade organics was noted for free volatile fatty acids, amino acids, carbohydrates, humic acids and fulvic acids, respectively.

Analyses of the different heavy metals in the leachate samples showed that high concentrations are generally present; iron and zinc were found in especially high concentrations. Determination of metals in leachate by flame atomic absorption spectroscopy was subject to large matrix interferences that were circumbented by use of the standard addition technique. Membrane fractionation analysis showed that the majority of the metals permeated the 500 MW UF membrane, indicating that chelation of most metals by refractory organics in leachate plays a minor role in metal attenuation processes; an exception was iron, most of which was associated with the 100,000 MW UF retentate. Biological treatability studies showed that the metal concentrations in leachate generally do not cause any toxic effects due to the formation of hydroxides and carbonates in aerobic systems and sulfides and carbonates in anaerobic systems.

This report was submitted in fulfillment of Contract No. 68-03-0162 by the University of Illinois under the sponsorship of the U.S. Environmental Protection Agency. This report covers the period June 30, 1972 to November 30, 1974, and work was completed as of September 1976.

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I

RECOMMENDATIONS FOR FURTHER RESEARCH

Considerable spread was observed in some of the results of grab sample analyses from different landfills when these analyses were correlated with the age of the landfill. The cause of these variations are suspected to be the individual characteristics of the different landfills and the local climatic conditions. Those factors affecting the trend should be more accurately defined to allow prediction of future impacts on the environment and the long-term efficacy of leachate treatment methods.

Additional, more specific chemical analysis of the organic fractions identified is recommended to ascertain the presence of environmentally significant organics and the presence of organics significant in leachate treatment.

Additional basic research is necessary to more clearly define the limitations of the different separation techniques such as membrane ultrafiltration and gelpermeation chromatography using naturally occurring organics of different molecular weight and chemical composition. This should be followed by extensive degradation and identification studies to more clearly define the chemical nature of each of the different molecular weight fractions.

II

CHARACTERIZATION OF SANITARY LANDFILL LEACHATES AND ITS SIGNIFICANCE TO LEACHATE TREATMENT

CONCLUSIONS

The present study conducted an extensive analysis of both organics and inorganics present in leachate samples collected from landfills located in different parts of the United States. Several ratios were determined for leachate such as COD/TOC, BOD_5/COD, VS/FS, and total carbon present in the free volatile fatty acids to TOC. These ratios reflected results of the extensive analysis of the organic matter in leachate and can be used to predict the effectiveness of either biological or physical-chemical treatment methods with a given leachate. Some of these ratios, such as COD/TOC, are also used as an internal check on the reliability of the results of chemical analysis of leachate samples.

When the organic analysis was related to the leachate treatability, it was noted that leachate collected from recently leaching landfills is best treated by aerobic or anaerobic biological treatment processes as they are most effective in removing the free volatile fatty acids that are present in large quantities. Physical-chemical treatment processes are most effective in treating leachate from stabilized landfills or to further remove organic matter in effluent of biological units treating leachate. Of all the physical-chemical processes evaluated, activated carbon and reverse osmosis gave the best removal of organic matter.

INTRODUCTION

The sanitary landfill method for the ultimate disposal of solid waste continues to be widely accepted and used. To minimize environmental insult, it is necessary that the selection of the site be given special attention and that the site be properly designed, constructed, and operated. After the solid waste is placed in the fill, problems may still develop. One such problem is caused by infiltrating rainwater and the subsuquent movement of liquid (leachate) out of the fill into the surrounding soil. This leachate often contains a high concentration of organic matter and inorganic ions, including heavy metals. A number of incidences have been reported where leachate has contaminated the surrounding soil and polluted an underlaying groundwater aquifer or nearby surface water. In one of the recent cases of groundwater contamination by leachate, private wells located 300 m downstream from the Llangollon landfill (completed and closed in 1968) in New Castle County, Delaware, were heavily polluted and subsequently abandoned (R. F. Weston Inc., 1974). The municipal water supply from Genesco, Illinois, was polluted by leachete from a garbage dump located about 500 m north of the well field, while private wells located about 200 m from a landfill in Kane County, Illinois, showed bacterial and chemical pollution (Walker, 1969). One way to avoid or correct such situations is to collect and treat leachate. Treatability of leachate, however, is related to its chemical composition, especially the nature of the organic matter.

The composition of leachate has been investigated in several studies. One of the first studies, reported by Merz (1952), was concerned with inorganic ions leached out of incinerator ash dumps. Other studies, published later, were conducted with leachate generated from sanitary landfills handling municipal solid waste (1954). Lysimeter studies, conducted by Qasim and Burchinal (1970), Fungaroli and Steiner (1971), Pohland (1972), and Pohland and Maye (1970), determined organic matter in addition to the inorganic constituents by analyzing the leachate for COD, BOD, free volatile fatty acids, organic-nitrogen, and in one study lignins and tannins.

An extensive chemical analysis of leachate generated from landfills located in different parts of the United States was conducted at the University of Illinois. Particular emphasis was given to detailed chemical analysis of both the organic constituents and heavy metals. Since the methods developed for organic analysis are elaborated and time-consuming (DeWalle and Chian, 1974), a number of parameters were identified to represent the more complex actual composition of organic matter in leachate. Laboratory-scale biological and physical-chemical treatment processes were evaluated for their ability to remove the individual contaminants in leachate. Leachate from the pilot-scale lysimeter installed at the University of Illinois was used throughout the treatability study.

MATERIALS AND METHODS

Representative leachate samples from a number of completed lysimeters and pilot- and field-scale sanitary landfills were carefully collected and stored at refrigeration temperature (5°C) for analysis. These sites are located in the United States at representative locations and cover a range of temperature and precipitation regimens (Table 1).

The gross properties of leachate, svch as chemical oxygen demand (COD), total organic carbon (TOC), pH, conductivity, oxidation reduction potential (ORP), turbidity, suspended solids (SS), volatile solids (VS) and nitrate (NO_3-N) etc., were immediately analyzed upon arrival in the laboratory according to procedures outlined in Standard Methods (American Public Health Association, 1971). These were followed by extensive analysis of the organic matter and heavy metals in leachate.

A unique and comprehensive fractionation scheme was developed for the concentration and separation of organic compounds of various molecular weights using ultrafiltration and reverse osmosis membranes and gel permeation chromatography. A Hewlett Packard 5750 B gas chromatograph equipped with a column containing a NPGS liquid phase was used for the analysis of free volatile fatty acids. The heavy metals were determined with atomic adsorption using a Beckman Unit, Model 485 (Fullerton, Ca.). It was found that any analytical methods relying on colorimetric techniques were undesirable with the undiluted leachate due to the interfering color contributed by leachate, unless extensive dilution of samples was practical (Chian and DeWalle, 1975). Standard addition methods were employed to determine the magnitude of the interfering effect.

The leachate used to evaluate its treatability by biological and physical-chemical processes was obtained from a laboratory lysimeter (1.52 m diameter by 3.04 m high) containing 1620 kg of shredded (3.8 cm grate

TABLE 1.—Description of Sites from Which Leachate Samples were Collected

Location (1)	Code (2)	Solid waste fill characteristics (3)	Date solid waste placed (4)	Date sample collected (5)	Weather Characteristics[a] Average temperature, in degrees Celsius (6)	Weather Characteristics[a] Rainfall, in millimeters (7)	Reference (8)
University of Illinois, Urbana, Ill.	UI	milled, rubber lysimeter	December 1972	February 1, 1973	10.9	1,002	This study
Dane Water Treatment Plant Madison, Wisc.	UNC	unmilled, covered, lysimeter	August 1972	November 20, 1973	7.2	780	Reinhardt and Ham (1973)
	MUNC	milled, uncovered, lysimeter	August 1972				
County of Sonoma, Santa Rosa, Calif.	SONCON	control, pilot fill	October 1971	July 9, 1973	7.8	153	County of Sonoma, Emcon Assoc. (1975)
	SONREC	recirculation, pilot fill					
Cincinnati Boone County EPA landfill, Ohio	CINC	unmilled, pilot fill	June 1971	October 2, 1973	12.7	1,007	Solid and Hazardous Waste Research Laboratory (1973)
Georgia Institute of Technology, Atlanta, Ga.	ATLCON	control, lysimeter	September 1971	December 20, 1973	16.4	1,230	Mao and Pohland (1973)
	ATLREC	recirculation lysimeter	September 1971				
Pennsylvania State University, College Park, Pa.	PACP67	unmilled, pilot fill	November 1967	February 17, 1973	8.1	980	Apgar and Langmuir (1971)
Drexel University, Kennett Square, Pa.	PDKS-C	unmilled, pilot fill	May 1966	February 15, 1973	12.3	1,118	Fungaroli (1971)
Wheaton Landfill Illinois	LW-17	field scale fill	December 1967	October 31, 1972	9.4	886	Hughes, et al (1971)
	LW-59	field scale fill	September 1959				
Dupage County Landfill Illinois	LW-59	field scale fill	February 1959				

[a] Source: U.S. Department of Commerce, National Oceanic and Atmospheric Administration, Environmental Data Service, National Climatic Center, Asheville, N.C.

opening) refuse obtained from the Hartwell section of Cincinnati, Ohio. It was compacted to 0.33 ton/m^3 (556.9 pound/yd^3). Water was applied daily to bring the solid waste to field capacity within 30 days, and thereafter an equivalent of 0.89 mm precipitation per week was added to generate sufficient leachate for evaluation of leachate treatment methods.

RESULTS AND DISCUSSION

The composition of leachate samples from different sanitary landfills, as reported in the literature, show a large variation. Table 2 summarizes these ranges of leachate composition; these data show that the age of the landfill, and thus the degree of solid waste stabilization, has a significant effect on the composition of leachate. Other factors that contribute to the variation of data are solid waste characteristics (e.g., the composition and size of the waste and degree of compaction), the moisture content and degree of rainwater infiltration, temperature, sampling technique, and analytical methods. Other factors such as landfill geometry and interaction of leachate with its environment prior to sample collection also contribute to the spread of data. Most of these factors are rarely defined in the literature, making interpretation and comparison with other studies difficult, if not rather arbitrary.

The time lapse between sample collection and analysis is one such factor that is seldom defined. Figures 1 and 2 depict changes in chemical oxygen demand (COD), turbidity, color (absorbance at 400 nm of the 1:10 diluted sample), pH, suspended solids (SS), oxidation reduction potential (ORP), and specific conductivity of leachate collected from the lysimeter at the University of Illinois. Analyses were performed immediately after sampling and frequently during storage at 5°C in a capped bottle for a twelve day period (17,280 minutes) during which time small aliquots were removed for analysis. It is seen from these figures that the ORP showed the greatest change and became more positive immediately after sampling. However, it stabilized after approximately one day (1440 minutes), indicating that this parameter should be determined immediately at the time of sampling. The other parameters such as SS, color, and turbidity started increasing in magnitude after approximately one day and, unlike ORP, they continued to increase with time. The sharp rise of these parameters shown in Figure 1 and 2 is amplified by the use of the logarithmic time scale. As expected, these changes were observed to occur more rapidly when the sample was exposed to aerobic conditions and stored at room temperature (Chian and DeWalle, 1975).

Table 2. Composition of Leachate from Different Sources as Measured by Different Researchers

Source / Parameter	SWARL (1970) (Cincinnati)	Fungaroli (1971) Drexel U.	[illegible] and Ham (1971)	Hughes et al. (1971) DuPage [illegible]	Hughes et al. (1971) DuPage [illegible]	Hughes et al. (1971) DuPage [illegible]	Hughes et al. (1971) DuPage [illegible]	Hughes et al. (1971) Winnetka [illegible]	Hughes et al. (1971) Winnetka [illegible]	Hughes et al. (1971) Winnetka [illegible]	Wang (1974) Riverside [illegible]
Time Span (year)	1	2	2.5	5.5	6.5	14.5	16.5	3.5	12.5	16.5	7.5
COD	16,000-22,000	1,000-47,200	2,770-10,650	8,000	2,940	380	40	4,360	475	350	
BOD_5	7,500-10,000		7,200-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
TOC											
pH	5.7-6.8	3.7-8.5	5.9-[illegible]	[illegible]			7.0				[illegible]
TS	12,000-14,000	0-42,000	4,020-7,[illegible]								
TDS	10,000-14,000			6,794	5,800	1,730	1,700	3,000	864	824	
TSS	100-700	10-26,500									
Specific Conductance	6,000-9,000										
Alkalinity ($CaCO_3$)	800-4,000	0-8,700	1,600	5,570	4,700	7,830	2,250	3,070	3,230	1,470	[illegible]
Hardness ($CaCO_3$)	3,500-5,000	0-5,000	1,400	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Total P	25-35	0-130	72	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Ortho-P	23-33										
NH_3-N		0-400									[illegible]
NO_3-NO_2-N	0.2-0.8			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Ca	900-1,700		80	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Cl	600-800	4.7-2,[illegible]	400	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Na	450-500	0-7,700		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
K	295-310			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Sulfate	400-650	25-[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Mn	75-125		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Mg	160-250			[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Fe	210-325	0-1,700	220	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Zn	10-30	0-[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Cu		0.9-[illegible]		[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Cd				[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Pb				[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	

NOTE: All figures in mg/l except Specific Conductance which is measured as µmhos/cm and pH as pH units

* Based on 20 Day BOD

** [illegible] value calculated from Mg and Ca [illegible]

± [illegible] only NO_3 analyzed, NO_2 not determined

Table 2 continued

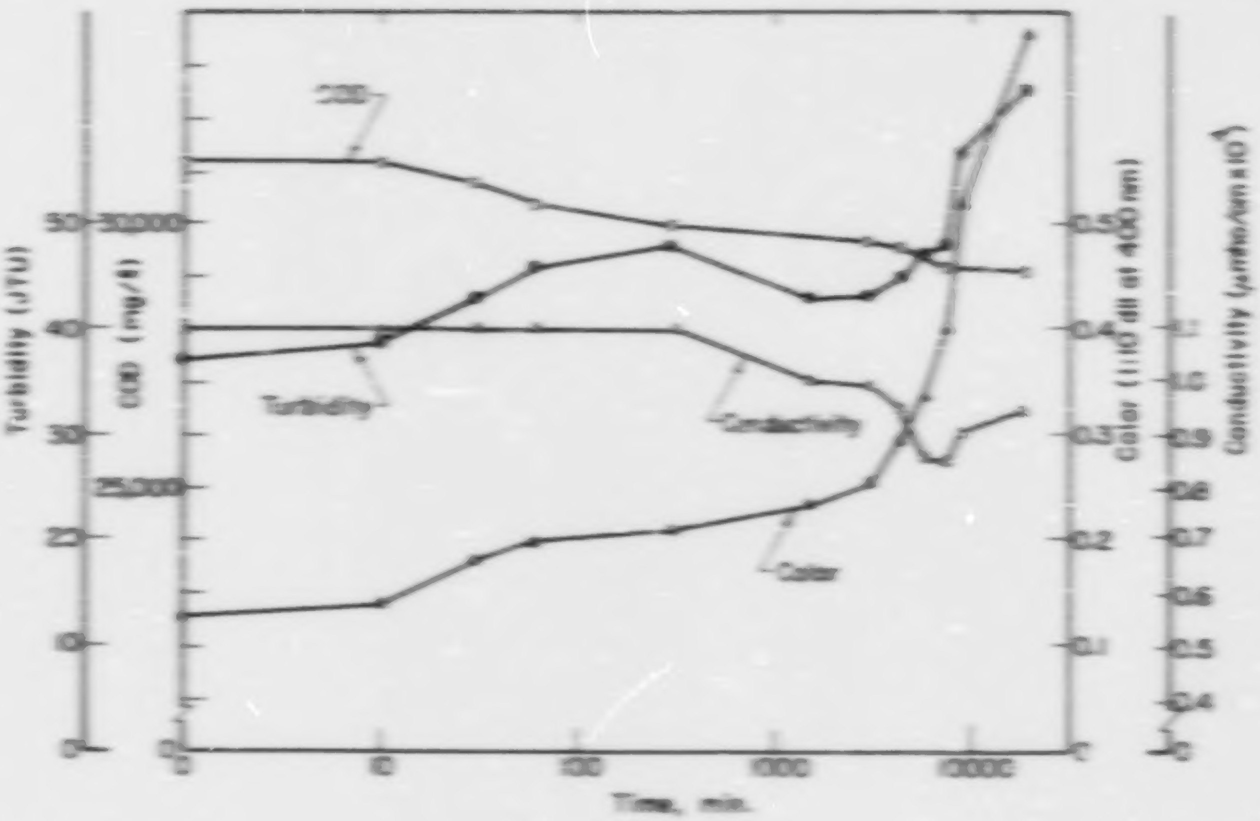

Figure 1. Changes in Chemical Oxygen Demand, Turbidity, Color, and Conductivity of Leachate During Storage at 5°C in a Capped Bottle

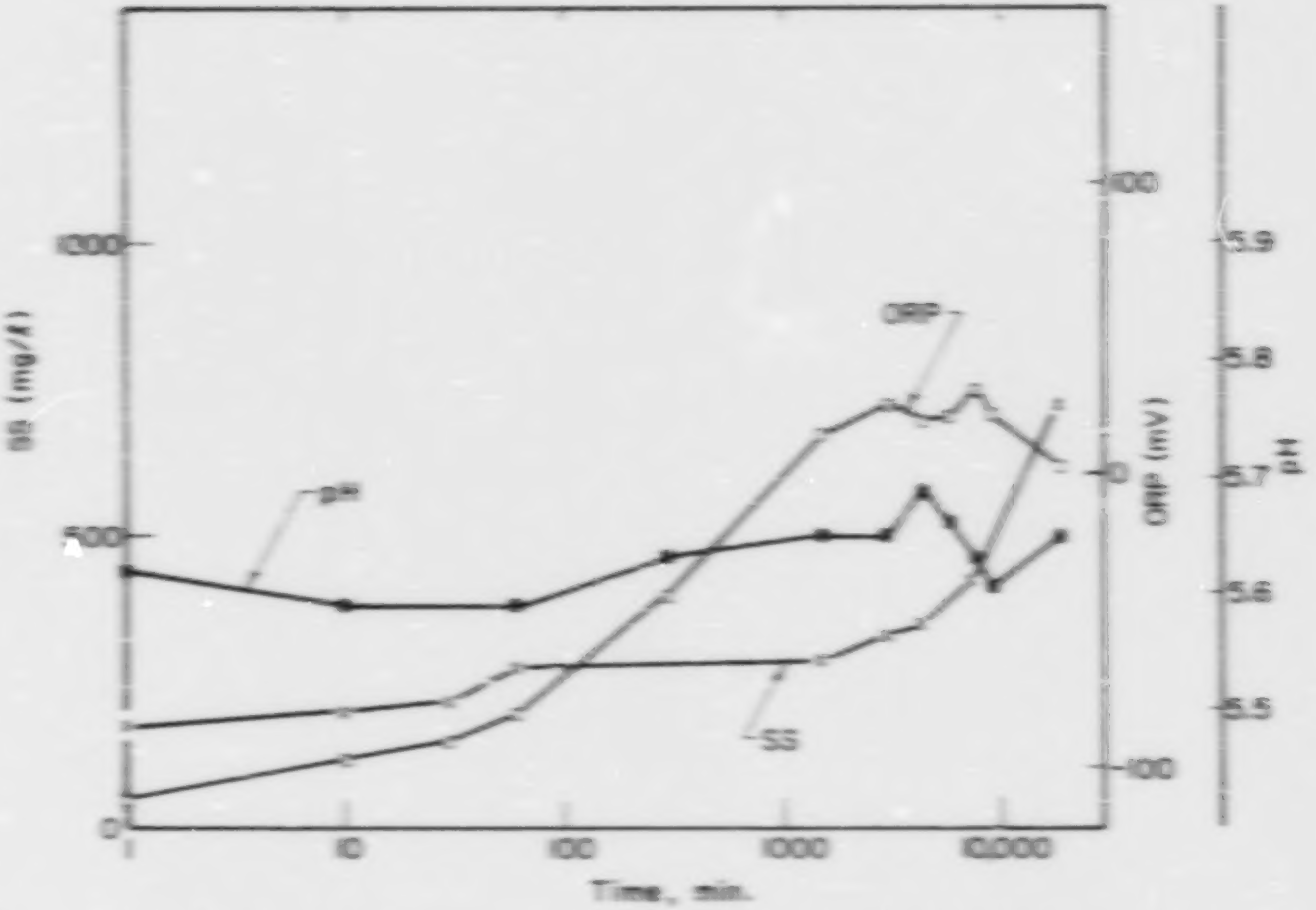

Figure 2. Changes in Oxidation Reduction Potential (ORP) pH and Suspended Solids (SS) of Leachate During Storage at 5°C in a Capped Bottle

The changes that took place with the color parameter corresponded to a visual change of the sample color from light yellow to dark brown, as reported by many other investigators, and are caused by the oxidation of the ferrous ion to the ferric form. The latter then forms ferric hydroxide colloids which contribute to the brownish color of leachate and the increase in suspended solids containing large quantities of iron. As a result of this, turbidity also increases with time of storage. Since a daily sample was taken from the container, exposure of the leachate remaining in the container to atmospheric oxygen is the major cause contributing to the decrease in COD and the increase in suspended solids. Storage under anaerobic conditions and at low temperature is therefore necessary. Thus, the necessity of establishing strict sampling procedures and testing schedules for leachate analysis is evident. Since the samples had to be collected from different parts of the United States, it was not practical to conduct on-site measurements; the most sensitive parameters however, were measured in the laboratory within 48 hours after collection. The remaining gross properties of leachate samples were analyzed within two weeks after sample collection while being stored at 6°C.

Results of chemical analysis of leachate samples collected from the different sites are presented in Table 3. It is seen from Table 3 that there is a wide variation in concentrations of organic matter in leachate as measured by the gross properties of COD, TOC, and BOD_5. Values of COD vary from 71,670 mg/l with leachate sample "MUNC" obtained from an 8-month old uncovered landfill containing milled refuse to 412 mg/l with sample "LW-17" obtained from an approximately 17-year old landfill. The concentration of inorganics as measured by fixed solids (FS) also

[illegible]

fluctuates from a high value of 22,895 mg/l to a low value of 812 mg/l. Other organic and inorganic contaminants also follow the trend of a decrease in strength with the increase in age of the landfills.

Since the concentration of the different pollutants will show a considerable variation on a day-to-day basis, it is more beneficial to use the ratios of different parameters instead of their absolute values. Further analysis of data in Table 3 reveals that many ratios of chemical properties, such as COD/TOC, BOD/TOC, VS/FS, SO_4/Cl, etc., reflect the composition of the organic matter in leachate and are in turn related to the age of the landfill. The parameter found to be useful in relating to the composition of organic matter was the ratio of chemical oxygen demand to total organic carbon (COD/TOC) (DeWalle and Chian, 1974).

The change in the COD/TOC ratio with the age of the landfills or lysimeters is shown in Figure 3a. These ratios were calculated and plotted from data shown in Table 3. It can be seen from Figure 3a that the COD/TOC ratio tends to decrease as the age of the landfill increases and this ratio varied from 3.3 for a relatively young fill to 1.16 for an old fill. The maximum possible COD/TOC ratio for several organic compounds is 4.0, and it can be as low as 1.3 for organics containing carboxyl groups (Rickert and Hunter, 1971). A decrease in this ratio in the leachate samples represents a more oxidized state of the organic carbon which becomes less readily available as an energy source for microbial growth. These organics are generally degradation products of microbial activity and increase as the age of the landfill increases. The resulting leachate thus becomes less amenable to biological treatment.

The decrease in COD/TOC ratio is reflected by a gradual change of the composition of the organic matter as measured with the BOD_5 or free volatile fatty acids test. Since the biochemical oxygen demand (BOD) test is predominantly a biological test, it generally reflects the biodegradability of the organic matter in leachate and is, therefore, in itself a direct

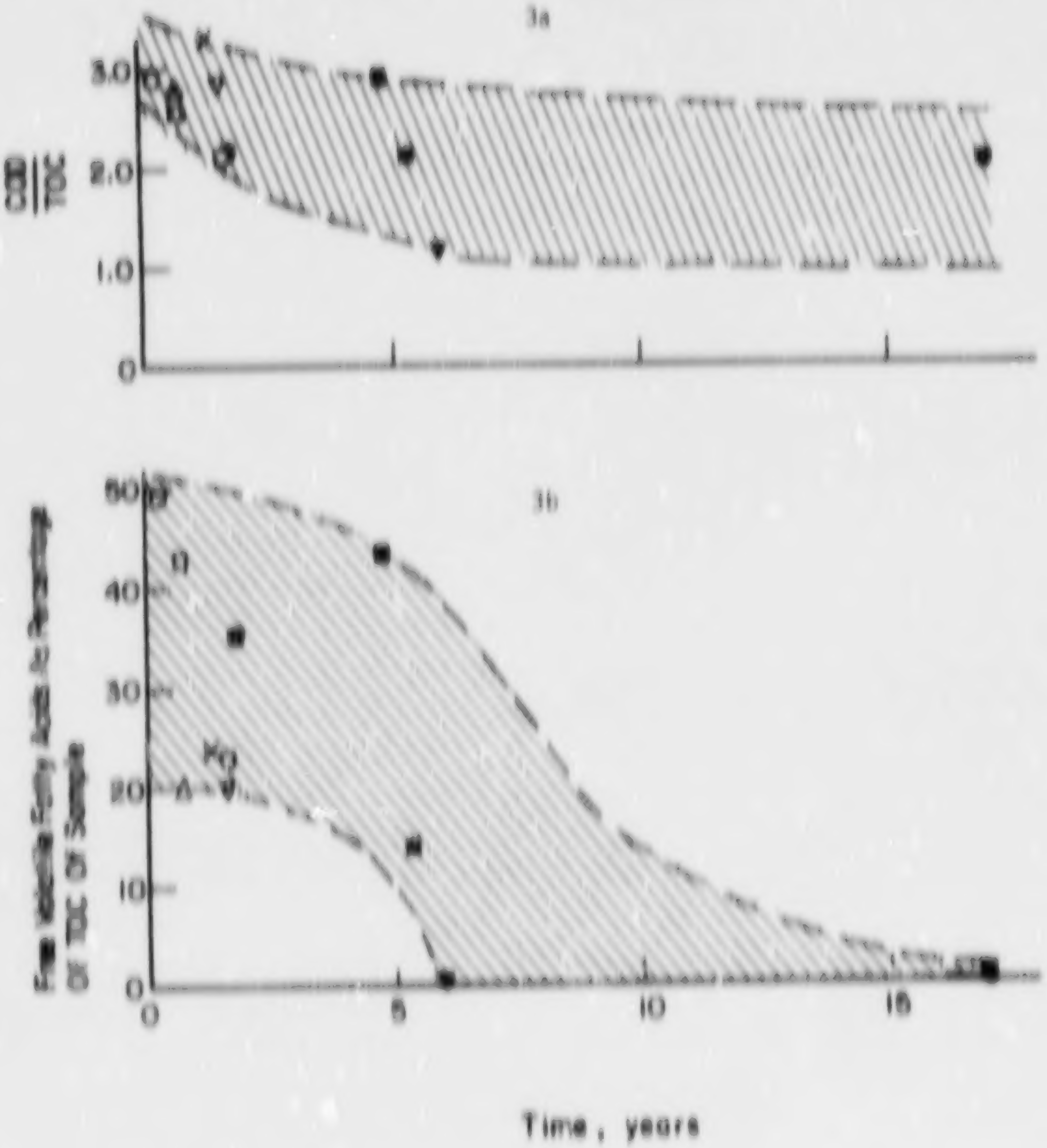

Figure 3. Changes of Ratios of COD/TOC and Free Volatile Fatty Acids as Percentage of TOC of the Sample Versus Age of the Landfill

measure of the treatability of leachate by biological processes. Similar to COD/TOC ratio, the BOD/COD ratio shows a decrease as the age of the landfills increase (Figure 3b). This relationship corroborates results from a recent study of Miller *et al*., (1974). The calculated ratio of BOD/COD based on his data showed a decrease from 0.47 to 0.07 within a period of twenty-three years. They are compared to a decrease from 0.8 to 0.048 as found in this study within a time span of seventeen years (Figure 3b). The BOD test, however, is subject to more variation than the TOC analysis, since the value obtained depends on the dilution of the leachate and the amount of seed added. Readily oxidizable inorganics such as Fe^{++} will contribute to both COD and BOD values; however, their effect was found to be relatively small. It is even further reduced by taking the ratio of the two parameters.

Specific organic analysis showed that the free volatile fatty acids constituted the main fraction present in leachate. Since free volatile fatty acids are readily biodegradable (Chian and DeWalle, 1975), a decrease in the ratio of carbon present in free volatile fatty acids to TOC corroborates the decrease in BOD/COD ratio. Figure 4b depicts the decrease of this ratio from 0.49 to 0.05 with increasing age of the landfill. Measurement of the individual volatile acids using GC, showed a relative increase in odd numbered fatty acids. The ratio of carbon present in the odd- and even-number volatile free fatty acids found in the present study increased from 0.27 to 0.55 during the first two-year period when appreciable amounts of fatty acids are present in leachate. Acetic acid is the main catabolic product produced by microorganisms during anaerobic fermentation. This is followed by condensation reactions to form other acids of higher molecular weight such as butyrate. The formation of odd numbered fatty acids occurs when repeated catabolic and anabolic metabolisms proceed in the presence of microorganisms responsible for producing them. The increase in the odd-number fatty acids, such as propionic and valeric has been reported by Burchinal (1970), Songonuga (1969) and Thompson (1969) to form at the later stage of leachate production.

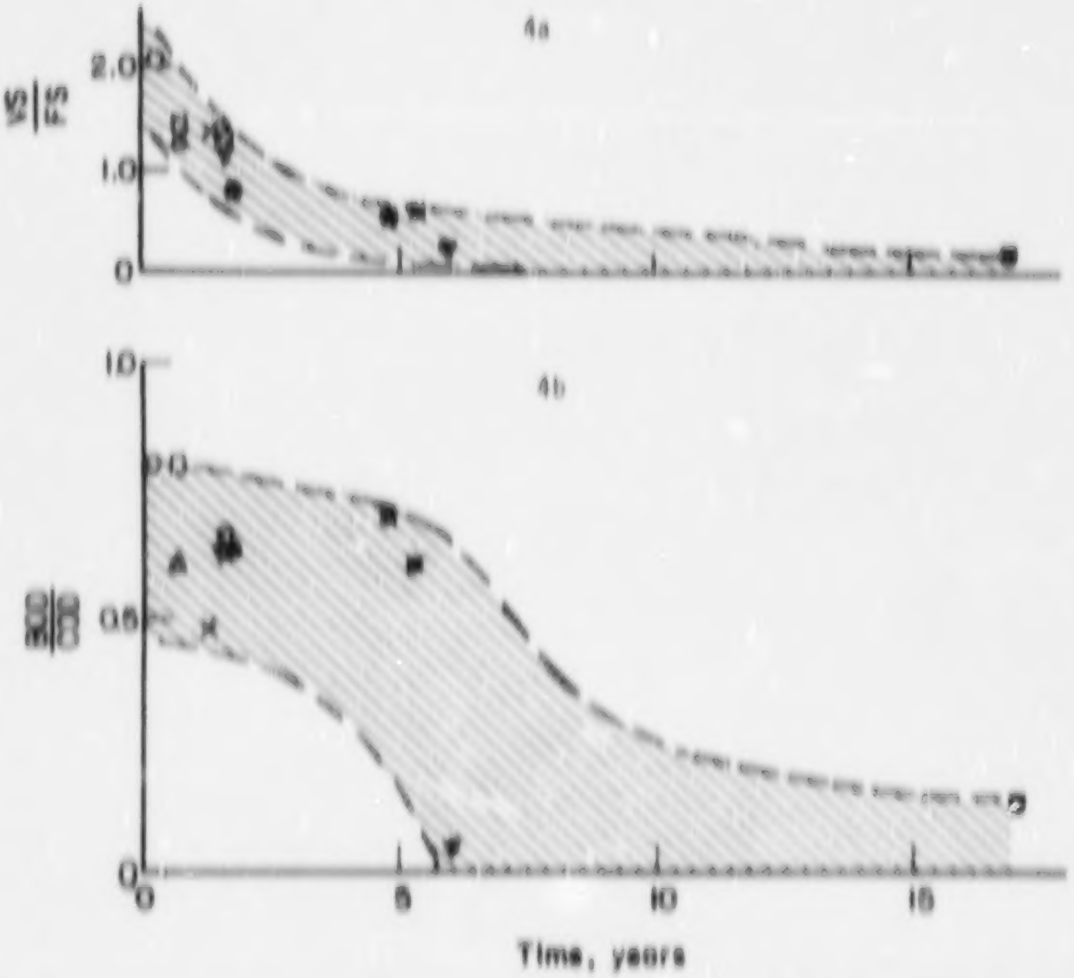

Figure 4. Changes of Ratios of Volatile Solids/Fixed Solids (VS/FS) and BOD/COD of Leachate Samples Versus the Age of Landfills

Due to its initially biodegradable nature, the organic compounds decrease more rapidly than the inorganics with increasing age of landfill. The decrease of organics is due to anaerobic methane fermentation and washout. The inorganics only decrease due to washout by infiltrating rainwater. The ratio of total volatile solids to total fixed solids (VS/FS) therefore decreases with the age of the fill (Figure 4a). It is seen that this ratio decreases gradually from a value of 2.0 for a relatively young fill to 0.2-0.25 for old landfills. Such a decrease was in corroboration to results of Qasim and Burchinal (1970). It was found that the calculated ratio of this parameter decreased from 2.06 to 1.64 and from 1.88 to 1.45 during a period of 121 days and 163 days with leachate obtained from simulated landfills having a depth of 3.5 m and 2.3 m, respectively.

Other parameters, such as sulfate to chloride ratio, oxidation reduction potential (ORP) and pH, also reflect the degree of stabilization of the landfill and thus the leachate generated from the fill. Figure 5a depicts the decrease of the ratio of sulfates to chlorides in leachate with respect to the age of the landfill obtained from this study. This rapid decrease is attributed to the decrease in concentration of sulfates as a result of anaerobic conditions prevailing in the landfill in which sulfate is reduced to sulfide. The latter is precipitated out with iron and other heavy metals. A similar decrease in this ratio was calculated from data reported by Pohland (1972) with leachate generated from both the control and the recirculating landfills. Since chlorides represent a nonbiodegradable parameter, it can be used to assess the extent of leachate contamination (Anderson and Dornbush, 1967).

The trend in the SO_4/Cl ratio is inversely related to the ORP parameter. The largest decrease in SO_4/Cl ratio corresponds with the lowest ORP values confirming the high degree of anaerobiosis in the landfill. The increase in ORP readings with time, as shown in Figure 5b, also reflects the degree of stabilization of leachate. Figure 5c shows the increase

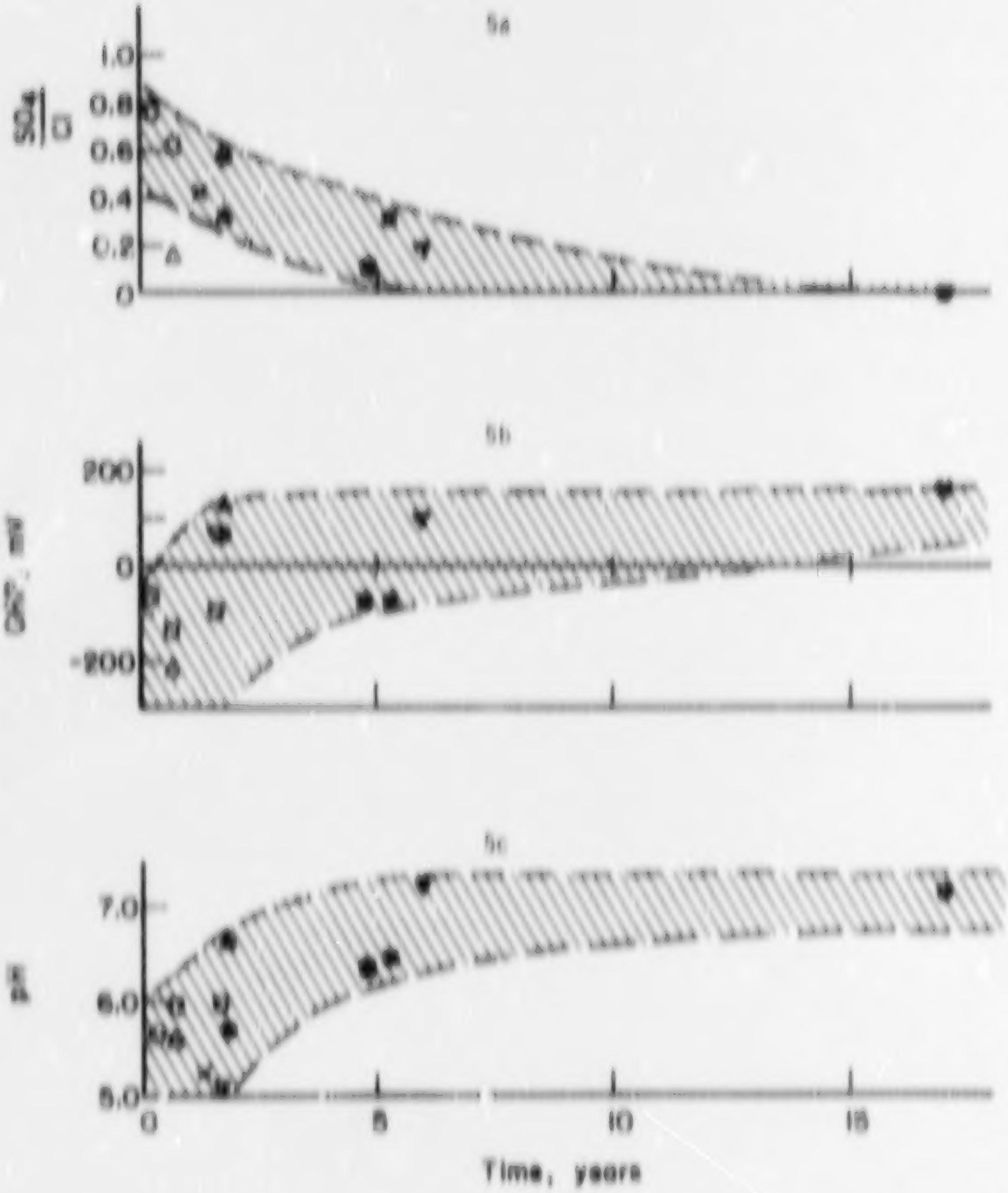

Figure 5. Changes of SO_4/Cl Ratio, Oxidation Reduction Potential (ORP) and pH of Leachate Samples Versus the Age of landfills.

in pH of leachate with time, which reflects the decrease of the concentration of the partially ionized free volatile fatty acids.

The potential use of these parameters is twofold. It can be used to establish a generalized trend between the leachate composition and the age of the landfill, and can be employed as an internal check on the consistency of the analytical results. The COD/TOC ratio, for example, can be used to determine the reliability of either the COD or TOC measurements. If the results of the analysis of a given leachate sample yield a ratio of COD/TOC greater than the theoretical maximum value of 4.0 for soluble organics, either the COD or TOC value is in question.

The ultimate goal of relating these parameters to the composition of organic matter and the age of the landfill, is, however, to establish useful criteria for the choice of specific treatment processes best suited for the removal of organic contaminants from leachate. Several investigators have studied the treatment of sanitary landfill leachate and promising results have been obtained with a number of treatment processes. Review of results of their studies indicates that the specific process suitable for the treatment of a given leachate is related to its chemical composition which is in turn related to the degree of stabilization of the refuse or the age of the landfill. The second phase of this study was therefore conducted to relate the performance of each treatment method to the composition of the organic matter in leachate. Since this approach is elaborate and time-consuming, several leachate parameters were selected to predict the effectiveness of each treatment process. Determining the value of these parameters and knowing the age of the landfill will enable the design engineer to select suitable treatment processes for the removal of organic matter in leachate.

Understanding the removal efficiencies of each treatment process requires a basic knowledge of the mechanisms resulting in the removal of specific organic fractions in leachate. Chian and DeWalle (1975b) studied the aerobic biodegradation of leachate collected from a lysimeter having an age of five months and showed four distinct phases of substrates utilization by microorganisms. Carbohydrates were found to be utilized in the first phase followed by uptake of fatty acids in the second phase. Catabolites, such as amino acids, accumulated during the active phase of acid metabolism, were found to be assimilated in the third phase. The humic carbohydrate-like materials, having a molecular weight greater than 50,000, which were accumulated in the third phase, were removed in the fourth phase. The resulting residual refractory organic matter remaining beyond the fourth phase consisted mainly of fulvic acid-like materials with a molecular weight predominantly between 500 and 10,000. DeWalle and Chian (1974) showed that the high-molecular-weight humic-carbohydrate fraction affected bacterial flocculation. This high-molecular-weight fraction was poorly removed by activated carbon treatment of the activated sludge effluent, while the low molecular weight fulvic acid-like materials were removed effectively (DeWalle and Chian, 1974b). Both the humic and fulvic substances are relatively inert to biological degradation. Based on these findings, it is anticipated that leachate generated from young fills in which the organic matter mainly consists of free volatile fatty acids, can be readily degraded by biological means. Leachate from old fills containing organics corresponding to the refractory material excreted by microorganisms in the later two phases of substrate utilization, is more amenable to physical-chemical treatment.

Aerobic biological treatment of leachate has been studied by many investigators as summarized in Table 4. Between 58 and 99% removal of COD was accomplished by either aerated lagoon or activated sludge systems. The range of BOD/COD of leachate fed to these systems varied between 0.45-0.81. All leachate samples employed in these studies were obtained from recently installed landfills and lysimeters. Leachate treatment using anaerobic digesters or anaerobic filters resulted in COD removals of 87 to 99% (Table 4). The BOD/COD ratio's of the leachate samples used for these studies ranged from 0.45 to 0.81. Results of the present

TABLE 4.—Result of Treatment Efficiencies Obtained in Different Biological Treatment Studies

Biological process (1)	Author (2)	Initial COD (3)	BOD/COD (4)	COD/TOC (5)	Treatment system (6)	Percentage COD removal (7)	Detention time (8)
Aerobic	Boyle and Ham (1974)	1,830	0.50	—	Aerated lagoon	74	5 d
	Cook and Foree (1974)	11,400	0.41	1.47	Aerated lagoon	91	10 d
	Karr (1972)	1,170	0.64	1.38	Aerated lagoon	77	8.5 d
	Pohland and Kay (1979)	300	0.71	1.76	Aerated lagoon	76	3.3 d
	Roy Weston Inc. (1975)	170	0.01	1.1	Aerated lagoon	0	7.7 d
	This study	10,000	0.61	1.7	Aerated lagoon	99	7 d
Anaerobic	Boyle and Ham (1974)	11,100	0.79	—	Anaerobic digester	91	10 d
	Foree and Reid (1973)	17,900	0.41	2.41	Anaerobic digester	97	10 d
	Karr (1972)	16,700	0.63	3.03	Anaerobic digester	99	15 d
		5,900	0.76	2.81	Anaerobic digester	95	10 d
	Rogers (1973)	1,500	0.81	—	Anaerobic filter using lime treated leachate	87	1.7 d
	This study	50,000	0.61	2.90	Anaerobic filter	97	27 d
Aerobic/Anaerobic	Boyle and Ham (1974)	—	0.18	—	Aerated lagoon treatment of anaerobic digester effluent	80	5 d
	Foree and Reid (1973)	110	—	3.33	Aerated lagoon treatment of anaerobic filter effluent	33	1 d
	This study	1,600	—	3.33	Aerated lagoon treatment of anaerobic filter effluent	17	7 d

study on treatment of leachate with activated sludge, aerated lagoon and anaerobic filter agree well with the above studies in that leachate collected from recently installed fills is amenable to both aerobic and anaerobic biological treatment.

Only one study tested the effectiveness of activated sludge treatment of leachate obtained from a 6-year old completed landfill (R. F. Weston Inc, 1974). Results of that study showed no decrease in COD after an aeration period of 184 hours due to the refractory nature of the organics as reflected by the ratios for COD/TOC and BOD/COD of leachate samples which were as low as 2.1 and 0.03, respectively. Thus, biological processes are not effective to remove organics in leachate from stabilized landfills that have been generating leachate for a substantial time period. A similar observation was made in the present study. It was found that the percentage COD removal in the anaerobic filter decreased with decreasing BOD/COD ratio's of the leachate from the laboratory-scale lysimeter.

These results are further substantiated by studies on aerobic treatment of effluents from anaerobic filters and anaerobic digesters treating leachate (Table 4). Boyle and Ham (1974) reported that only 40% removal of the COD material in effluent from the anaerobic digester was accomplished by polishing it with an aerobic process. The BOD/COD ratio of the effluent from the anaerobic digester was 0.18 as compared to 0.80 for the untreated leachate. Poor COD removal in effluents from anaerobic filters when treated with aerobic biological processes was also experienced by Foree and Reid (1973) and DeWalle and Chian (1976) (Table 4). Comparison of the COD/TOC and BOD/COD ratios of effluent from anaerobic units treating leachate with those determined in leachate from landfills having different ages (figure 3a and 3b) shows that the effluent of anaerobic processes is comparable to leachate from landfills of intermediate age. Since a substantial part of the biodegradable organic matter is already removed in the landfill, biological treatment methods are only moderately effective in removing the remaining organic matter in leachate.

No systematic studies have been conducted using anaerobic lagoons. Nordstedt (1970) observed a 70% BOD removal in the holding pond having an influent BOD of 778 mg/l. Davies (1973), however, only observed an 11% BOD reduction at a detention time of 12.5 days. These results therefore tend to indicate that biological processes are only effective when the leachate is in constant contact with the bacterial solids. None of the studies indicated the need to remove heavy metals prior to biological treatment. Formation of metal hydroxides in aerobic systems and formation of metal sulfides under anaerobic conditions will effectively eliminate the majority of the heavy metals present in leachate.

Several studies have tested the effectiveness of physical-chemical treatment of both raw leachate and biologically treated leachate samples. Chemical precipitation of leachate from a recently leaching landfill was reported by several investigators (Table 5) and a COD reduction of 0 to 40% in leachate having BOD/COD ratio's of 0.04 to 0.75. In spite of relatively low COD removals, chemical precipitation was found by most investigators to be very effective in removing color and iron from leachate. However, relatively large dosages of lime were required (Table 5).

The above results can be explained by a related study reported by Chian and DeWalle (1976) which showed that lime treatment predominantly removed the fraction of organic matter having a molecular weight larger than 50,000. This fraction is initially present in relatively low concentrations in young leachate and almost absent in old leachate. Since the 50,000 molecular weight fraction was found to increase and then decrease during the last phase of substrate utilization, lime treatment will be most effective with leachate from medium aged

TABLE 6 — Results of Treatment Efficiencies Obtained in Different Physical-Chemical Treatment Studies

Treatment process (1)	Author (2)	Initial COD (3)	BOD/COD (4)	COD/TOC (5)	Treatment system (6)	Percentage COD removal (7)	Dosages (8)
Chemical Precipitation	Cook and Foree (1973)	[illegible]	[illegible]	[illegible]	Lime	[illegible]	[illegible]
	Ho et al (1974)	[illegible]	[illegible]	—	Ferric chloride	[illegible]	[illegible]
		[illegible]	[illegible]	—	Alum	[illegible]	[illegible]
		[illegible]	[illegible]	—	Lime	[illegible]	[illegible]
		[illegible]	[illegible]	—	Lime treatment of anaerobic digester effluent	[illegible]	[illegible]
		[illegible]	[illegible]	—	Lime treatment of anaerobic digester effluent polished by aerated lagoon	[illegible]	[illegible]
	Karr (1972)	[illegible]	0.46	[illegible]	Alum and lime	[illegible]	[illegible]
					Ferrous sulfate	[illegible]	[illegible]
	Roy Weston Inc	[illegible]	[illegible]	[illegible]	Lime	[illegible]	[illegible]
	Rogers (1973)	[illegible]	[illegible]	—	Lime	[illegible]	[illegible]
	Simensen and Odegaard (1970)	[illegible]	[illegible]	[illegible]	Lime and aeration	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	Lime and alum	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	Alum and alum	[illegible]	[illegible]
	Thornton and Blanc (1973)	[illegible]	[illegible]	—	Lime	[illegible]	[illegible]
		[illegible]	[illegible]	—	Lime	[illegible]	[illegible]
	Van Fleet et al (1974)	[illegible]	[illegible]	—	Alum	[illegible]	[illegible]
	This study	[illegible]	[illegible]	[illegible]	Lime	[illegible]	[illegible]
Activated Carbon and Ion Exchange Adsorption	Cook and Foree (1974)	[illegible]	[illegible]	[illegible]	Activated carbon batch treatment of aerated lagoon effluent	[illegible]	
		[illegible]	[illegible]	[illegible]	Activated carbon column treatment of lime pretreated leachate	[illegible]	[illegible]
	Ho et al (1974)	[illegible]	[illegible]	—	Activated carbon batch	[illegible]	[illegible]
		[illegible]	[illegible]	—	Activated carbon column	[illegible]	[illegible]
	Karr (1972)	[illegible]	0.46	[illegible]	Activated carbon batch	[illegible]	[illegible]
	Pohland and Kang (1975)	[illegible]	[illegible]	[illegible]	Carbon batch treatment of activated sludge effluent	[illegible]	[illegible]
		[illegible]	[illegible]	[illegible]	Ion exchange treatment of activated sludge effluent	[illegible]	[illegible]
	Roy Weston Inc	[illegible]	[illegible]	[illegible]	Activated carbon batch	[illegible]	[illegible]
	Van Fleet et al (1974)	[illegible]	[illegible]	—	Activated carbon column treatment of leachate	[illegible]	

TABLE 6—Continued

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
					Activated carbon column treatment of alum pretreated leachate	53	
	This study	133	0.65	2.89	Activated carbon column treatment of leachate	70 decreased to 11 after 180 [illegible]	
		186	<0.1	2.33	Activated carbon column treatment of effluent of aerated lagoon	75	
		127	<0.1	2.86	Ion exchange column treatment of effluent of aerated lagoon	51	
		811	—	2.9	Activated carbon column treatment of effluent of anaerobic filter	98	
		133	<0.1	2.7	Activated carbon column treatment of aerated effluent of anaerobic filter	70	
Chemical Oxidation	Cook and Foree (1974)	115	0.07	2.17	Chlorination	11	61 mg bleach/l carbon
	Ho et al (1974)	1,790	0.71	—	Chlorination with calcium hypochlorite	7	8,000 mg/l Ca(ClO)₂ after 2 hr
		1,163	0.71	—	Ozonation	11	8 hr, 1,700 mg O₃/l · hr
	Karr (1972)	8,000	0.66	2.73	Chlorination	12	2,000 mg/l Cl₂
	Roy Weston Inc	115	0.08	2.1	Chlorination with calcium hypochlorite	0	1,000 mg/l Ca(ClO)₂
		128	0.06	2.1	Ozonation	22	4 hr 18 mg O₃/l · hr
	This study	1,230	—	2.8	Ozonation of anaerobic filter effluent	17	1 hr, 400 mg O₃/l · hr
		62	—	1.3	Ozonation of aerated lagoon effluent	44	1 hr, 300 mg O₃/l · hr
Reverse Osmosis	Roy Weston Inc	261	—	1.1	Reverse osmosis	60	90% Permeate yield
	This study	11,150	0.63	7.89	Reverse osmosis of leachate at pH 7.1, cellulose acetate membrane	76	78% Permeate yield
		11,320	0.61	7.89	Reverse osmosis of leachate at pH 8.0, cellulose acetate membr.	83	78% permeate yield
		668	—	1.9	Reverse osmosis of anaerobic filter of Nylon (DuPont B-9)	38	77% permeate yield
		156	—	1.1	Reverse osmosis of aerated lagoon of Nylon, cellulose acetate membrane	11	76% permeate yield

fills. Low COD removals are therefore observed at both high and low BOD/COD ratio's while it is largest for intermediate BOD/COD ratio's. This mechanism is further supported by results of Ho et al. (1974) who showed that the COD removal improved from 3 percent to 29 percent when the leachate was pretreated by aerobic processes.

Activated carbon treatment of raw leachate generally gives better removal of organic matter than is observed with chemical precipitation (Table 5). Using relatively large dosages of activated carbon, between 34 percent to as high as 85 percent COD removals were obtained (Table 5). Column studies with activated carbon resulted in COD removals of 59 to 94 percent. However, the column effluent was only evaluated for the first few bed volumes. A carbon column study of larger duration conducted in this laboratory with a leachate having a COD/TOC ratio of 2.89, showed an initial TOC removal of 70 percent which decreased to 13 percent after 140 bed volumes indicating that treatment of leachate by activated carbon is not feasible due to the large quantities of activated carbon required.

The above studies were conducted using leachate obtained mostly from young fills. Since free volatile fatty acids are the main components in such leachate, the overall removal of organics by activated carbon is not expected to be as high as compared with the more efficient biological methods discussed previously. Kipling (1965) reported that activated carbon removal of acetic acid was never greater than 20 percent. A recent study reported by Giusti et al. (1974) showed that the percentage reduction by activated carbon of acetic, propionic and butyric acids was 24, 33 and 60 percent, respectively,at a dosage of 5,000 mg/l. Although carbon adsorption increased as the homologous series of acid in aqueous solution is ascended (Traubes' rule), valeric and caproic acids are not present in large quantities in leachate. The variations in reported data on COD or TOC removal by activated carbon in young leachate are therefore attributable to both the varying proportion of low and high molecular weight free volatile fatty acids and the varying magnitude of the volatile fatty acid fraction as observed by Burchinal (1970) Songanuga (1969) and in the present study.

Since the effluent of biological units treating leachate is comparable in organic matter composition to leachate from an intermediate aged landfill, the results obtained in studies treating such effluent with activated carbon can be used to predict the effectiveness of activated carbon treatment of leachate from intermediate aged fills. Cook and Foree (1974) and Pohland and Kang (1975) found that activated carbon resulted in higher COD removals from biologically pretreated leachate than that from the untreated leachate (Table 5). The present study showed that organic matter removal by activated carbon increased with increasing biological stabilization or decreasing COD/TOC ratio's. Increased adsorptive capacities were found to correspond with leachate having relatively low free volatile fatty acid fractions.

Only one study reported COD removal by activated carbon with old stabilized leachate having a BOD/COD ratio of 0.04 (R. F. Weston Inc., 1974). It was found that activated carbon was able to reduce 86 percent of the COD. The reported surface concentration was 0.17 mg COD/mg activated carbon which is high as compared to values obtained for free volatile fatty acids. DeWalle and Chian (1974b) have shown that the major organic fraction removed by activated carbon was a fulvic acid-like material having a molecular weight (MW) between 500 and 10,000. Since, this MW fraction increases as percentage of the organic matter as the age of the landfill increases, activated carbon is expected to be more effective in treating biologically stabilized leachate.

Extensive studies have been reported on the use of oxidants as a means of removing organic matter in leachate. COD removals varying between 0 and 48 percent (Table 5) were obtained. Results by Ho et al., (1974) indicated that calcium hypochlorite produced the best results among all the oxidants evaluated, such as chlorine, potassium permanganate and ozone. Ozonation of leachate from an old fill was reported by R. F. Weston Inc, (1974) which showed a COD removal of 22 percent after a four-hour test. All tests, however, showed that the use of oxidants was less effective than activated carbon in removing the organic matter from such stabilized leachate. Ozonation of leachate from a recently generating landfill is also not promising because of strong resistance of fatty acids, especially acetic acid, to ozone.

Of all the physical-chemical methods evaluated in the present study, reverse osmosis membrane treatment was found to be most effective in removal of COD. Between 56 and 70 percent removal of TOC was obtained with the conventional cellulose acetate membrane while the removal increased to 85 and 88 percent with the polyethylenimine, NS-100, membrane at 85 percent product water recovery and 40.8 atm. Since low rejection of undissociated fatty acids by membranes was responsible for the leakage of TOC into the permeate (Chian and Fang, 1973), the performance of the membrane process was improved to 85-88 and 93-94 percent respectively when the pH of the leachate was increased from 5.5 to 8.0. Good TOC removal with cellulose acetate membranes was also reported by R. F. Weston Inc, (1974) with an old leachate having an average BOD/COD of 0.05 and COD/TOC less than 2. In addition to efficient TOC removal, removal of total dissolved solids was as high as 99 percent. However, severe membrane fouling was experienced with leachate and biological pretreatment of leachate prior to membrane processes is necessary.

The studies summarized above clearly show that the use of physical-chemical treatment processes treating young leachate does not produce the degree of organic removal that can be accomplished with biological processes. However, good results are observed with stabilized leachate collected from old fills. Similar good results are obtainable with leachate which has been stabilized biologically with both anaerobic and aerobic processes. Table 6 summarizes the proposed treatment processes for leachate as characterized by four parameters that have to be known, i.e., COD/TOC and BOD/COD ratios, absolute COD concentration and age of the fill. Using these values, a first approximation can be made to select proper treatment processes for the removal of organic matter present in leachate. However, if there are less than four such parameters agreeable to each other, more uncertainty is expected in the selection of the proper treatment processes.

Since only a limited number of leachate samples were analyzed in the present study, it was difficult to give the equivalent age of the so-called young, medium, and old fills to indicate at what age of the landfill the physical-chemical methods become less effective than the biological methods. A larger number of leachate samples from landfills having different ages should therefore be analyzed for these parameters in relation to their treatability. However, the results in Figures 3 and 4 tend to indicate that the leachate, which is amenable to biological treatment, is generated from the first five years during the life of the landfill, while the intermediate phase corresponds to an age of five to ten years. Leachate generated after approximately ten years is best treated by physical-chemical methods. These ranges of landfill ages are very tentative, and will depend on such factors as the duration of landfill construction, time for the fill to reach field capacity, density of the refuse, etc. The present conclusions are based on leachate samples collected from widely different fill conditions, and therefore should be representative of most situations.

TABLE 5.—Proposed Relationship between COD/TOC, BOD/COD, Absolute COD, and Age of Fill To Expected Efficiencies of Organic Removal from Leachate

Character of Leachate				Effectiveness of Treatment Processes						
COD/TOC (1)	BOD/COD (2)	Age of fill (3)	COD, in milligrams per liter (4)	Biological treatment (5)	Chemical precipitation (lime dose) (6)	Chemical oxidation (Ca (OCl)$_2$) (7)	O_3 (8)	Reverse osmosis (9)	Activated carbon (10)	Ion exchange resins (11)
>2.8	>0.5	Young (<5 yr)	>10,000	Good	Poor	Poor	Poor	Fair	Poor	Poor
2.0-2.8	0.1-0.5	Medium (5 yr-10 yr)	500-10,000	Fair	Fair	Fair	Fair	Good	Fair	Fair
<2.0	<0.1	Old (>10 yr)	<500	Poor	Poor	Fair	Fair	Good	Good	Fair

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III

THE USE OF MEMBRANE ULTRAFILTRATION AND SPECIFIC ORGANIC ANALYSIS FOR CHARACTERIZATION OF ORGANIC MATTER IN LEACHATE

CONCLUSIONS

Membrane ultrafiltration, gelpermeation chromatography, and specific organic analysis were used to separate and determine the main classes of organics present in variable strength leachate having COD values ranging from 81 to 71,680 mg/l. Ultrafiltration (UF) of a leachate sample obtained from an experimental lysimeter showed that 72.8% of the organics permeated the 500 MW UF membrane, and further analysis showed that most of these were present as low molecular weight free volatile fatty acids. Comparison of the gelpermeation TOC distribution with the TOC in the retentate of the UF membranes indicated that the nominal molecular weight estimated from the membrane ultrafiltration is approximately four times higher than the absolute molecular weight obtained with the former method. Evaluation of the serial UF procedure, using either membranes of stepwise increasing pore sizes or stepwise decreasing pore sizes, showed that the presence of high molecular weight organics and ferric hydroxides will decrease the molecular weight cutoff of a membrane. The nominal molecular weight cutoff of a membrane can change by a factor of ten as a result of the sequence during the ultrafiltration. It was therefore concluded that the parallel UF procedure resulted in a more representative separation than the serial procedure.

Further UF separation and gelpermeation followed by specific organic analysis and IR identification of the membrane-separated organics showed that the next largest organic fraction was a fulvic-like material with a relatively high carboxyl and aromatic hydroxyl group density. A small percentage of the organics (i.e., 6%) consisted of a high molecular weight humic-carbohydrate-like complex also characterized by a significant amount of hydrolyzable amino acids.

A carbon balance of the organics present in each fraction showed that 86% of the carbon in the 500 MW UF permeate and 50% of the carbon in the high molecular weight fraction was identified. Only 20% of the fulvic-acid-like fraction was identified, indicating that it consisted of complex molecular structures. Solvent extraction of the different membrane fractions showed the presence of esterified fatty acids associated with the high molecular weight humic fraction.

Membrane fractionation of leachate samples collected from different landfills showed a decrease of free volatile fatty acids in the 500 MW UF permeate with increasing age of the landfill. Further membrane fractionation showed that the high molecular weight humic-carbohydrate-like organics experience a more rapid decrease than the intermediate molecular weight fulvic-like organics.

Comparison of the distribution of specific organics in the different molecular weight fractions with the results obtained by other researchers with organics obtained from widely different environments shows a large similarity, indicating that universal bacterial processes are governing the character of these organics. The present study measured a relatively small amount of high molecular weight organics as compared to other studies. This is likely a result of the anaerobic environment during the leaching process which enhances acid fermentation of complex high molecular weight organics. The present study characterized stabilized landfill leachate by relative organic distributions of the following three groups: fatty acids < carbohydrates, proteins, and humic-like substances < aromatic hydroxyl, carboxyl and fulvic-like substances. This pattern reflects increased resistance of the organics to bacterial degradation.

INTRODUCTION

Major complications generally arise in the identification of general classes of organic matter present in wastewater, surface water and groundwater, due to the complex nature of these compounds. Many investigators have studied the techniques for concentration, separation and identification of different classes of organics. For example, Hunter and Heukelekian (1965) used primarily solvent extraction techniques to obtain fractions such as lipids, fatty acids, detergents, phenols, carbohydrates and lignins in domestic wastewaters. The magnitude of each fraction was then determined using gravimetric, colorimetric and chromatographic methods. When the organic matter concentrations in the water are low, a preconcentration step is generally employed. Myrick and Ryckman (1963), for example passed 20,000 l of river water through an activated carbon column, whereupon, the dried carbon was extracted with chloroform, ethanol, acetone and benzene.

Extraction or adsorption techniques are generally not effective in concentrating polar organics; these can, however, be concentrated by freeze drying, evaporation or distillation. The separation of such polar organic compounds is generally more cumbersome. Polar volatile organics can be detected by gas chromatographic techniques, while polar non-volatile organics, often having high molecular weights, can be separated by fractional precipitation, gelpermeation chromatography or membrane ultrafiltration. Rebhun and Manka (1971), for example, have determined humic acid gravimetrically after precipitation at pH 2 and fulvic acid by extraction in butanol. Gjessing and Lee (1967) used evaporation under reduced pressure followed by gelpermeation chromatography to obtain different organic fractions separated according to molecular size. Physical or chemical analysis was conducted only after the separation step was completed.

A disadvantage of the above methods is that only relatively small amounts of organic matter are obtained. For that reason DeWalle and Chian (1974) employed bench-scale membrane reverse osmosis and ultrafiltration units to obtain relatively large amounts of organics of different molecular sizes. After the membrane fractionation step, further separation was realized using gelpermeation chromatography followed by functional group analyses.

SEPARATING PROPERTIES OF MEMBRANES

The molecular properties of membranes have been recognized for more than 100 years in dialyses, ultrafiltration and reverse osmosis processes (Ferry, 1936) but have not found wide application until recently. Black and Christman (1963), for example, used dialysis membranes with pore sizes of 35 Å, 48 Å and 100 Å to obtain different fractions of colored organics present in surface water. Using ultrafiltration membranes with different molecular size cutoffs, Gjessing (1973) and Schindler and Alberts (1974) studied the molecular weight distribution of organics in surface waters. Fang and Chian (1975) employed reverse osmosis to concentrate and separate organics having relatively low molecular weights.

Dialysis is based on diffusion in which relatively small solutes permeate the membrane, while larger solutes are retained due to the seiving effect of the membrane (Kesting, 1971). Ultrafiltration separates from a solution those organics whose molecular dimensions are ten or more times larger than the solvent, by pressurizing the solvent to flow through a membrane (Michaels, 1968). The solute transport during ultrafiltration occurs by viscous flow mechanisms rather than diffusive mechanisms such as is the case in reverse osmosis. Because of these different transport mechanisms the rejection efficiency of ultrafiltration membranes is generally independent of the applied pressure, while the rejection for reverse osmosis membranes increases to an asymptotic maximum with increasing pressure (Michaels, 1968). Some deviations of this general behavior have been noted. For example, the rejection in membrane ultrafiltration may decrease beyond a certain pressure due to elastic distortion of the membrane while it may increase with increasing solute concentration due to higher solute drag on the pore walls. As a result of such viscous drag, the flux will also decrease. The occurrence of concentration polarization (i.e., the formation of a boundary layer of a substantially higher concentration than the bulk retentate concentration) will decrease the apparent rejection of the membrane. Such concentration polarization will generally not decrease the flux through the membrane due to the low osmotic pressure of the relatively high molecular weight organics (Blatt et al., 1970). Formation of a gel upstream of the membrane with a lower porosity than the membrane will increase the rejection (Michaels, 1968).

Craig et al. (1957) have concluded that molecular size of the solute is the main factor determining its permeation and that charge effects play a less significant role in membrane ultrafiltration. Mathematical expressions accounting for the steric hindrance to entry into a pore, the viscous drag at the wall of the assumed capillaries in the membrane and the friction between the solute and the walls of the capillary, indicate that the probability of permeation rapidly decreases when the solute diameter becomes more than half of the pore diameter. The greatest selectivity occurs when the solute diameter is 20 to 30% of the pore diameter (Renkin, 1954).

DIALYSIS MEMBRANES

The earliest membranes consisted of animal derived material, but were later replaced by synthetic materials such as cellulose (cellophane, cuprophane) or nitrocellulose (collodion, gradocol) membranes. The pore size of the first manufactured cellophane membranes (Visking Division, Union Carbide, Chicago, IL) was generally 40-60 Å (McBain and Kistler, 1931) but was later reduced to 20 Å (Morton, 1935). Thereafter, it was increased to 30 Å (Seymour, 1940; Madras, 1949) and presently varies between 30 Å (Renkin, 1954) and 46 Å (Durbin, 1960). The pore size of uncoated cellophane (Dupont, Stillwater, DE) generally varies between 62 Å (Renkin, 1954) and 82 Å (Durbin, 1960).

The cellulose membranes from Visking Company (Chicago, IL) have a stated pore size of 48 Å and are reported to retain organics with a molecular weight above 12,000. The molecular weight cutoffs, however, have been found to vary by a factor of ten among the different inflated diameters of the seamless tubing (Craig *et al.*, 1957). Cellulose membranes with molecular-weight cutoffs of 12,000, 6,000 and 3,500 are also commercially available (Spectrum Medical Industries, Los Angeles, CA). Several investigators have used cellulose membranes to concentrate and separate organics in wastewater and surface waters (Table 7), using pore sizes of 48 Å (Black and Christman, 1963), 40 Å (Gjessing, 1971), 25 Å (Werschaw and Burcar, 1967) and 24 Å (Packham, 1974; Dor, 1975). Several studies in Europe using LKB ultrafilters (LKB Comp., Upsola, Sweden) and were able to obtain organics with a molecular weight larger than 30,000 (Povoledo and Gerletti, 1968).

A wider range of pore sizes can be obtained using collodion dialyses membranes. Elford (1931) made ether alcohol collodion membranes with reproducible and well-defined pore sizes ranging from 100 Å to 30,000 Å, with the former pore size able to retain bovine serum albumin (MW 65,000). Elford and Ferry (1936) made a collodion membrane with a pore size of 80 Å able to retain ovalbumin (MW 45,000). A membrane with a pore size of 20 Å was able to retain sucrose (MW 342) as reported by Ferry (1936). The pore size measured by these researchers using Poiseuille's law, however, are about twice as large as the actual size (Renkin, 1954) since they did not take into account the viscous drag and the presence of blind pores. Using special curing steps collodion membranes can be made that are able to retain glucose (MW 180) but allow passage of urea (MW 60) (Carr, 1959). Most collodian membranes have pore sizes of 100 to 150 Å (Meschia and Setnikar, 1958), 60 to 80 Å (Jacobs, 1972) and approximately 30 Å (Robbins and Mauro, 1960). Membranes with molecular weight cutoffs of 20,000, 65,000, 70,000, 80,000 and 160,000 are commercially available (Schleicher and Schuell, Keene, NH). Black and Christman (1963) used a collodion membrane with a pore size of 35 Å to concentrate and separate organics in surface water.

Table 7. Dialysis Studies Characterizing the Molecular Weight Distribution of Aquatic Organics

Author	Source	Membrane	Percentage Retained (%)	Parameter	Organic Matter Concentration (mg/l)	Parameter
Barth and Acheson (1962)	tap water	cellulose dialysis membrane [illegible] MW ~ 10,000			0.08	Poly-saccharide
Black and Christman (1963a)	surface water	collodion membrane 0 350Å	91	color	142	COD
		cellophane membrane 2,400	87.5	color	142	COD
		membrane filter 0 100Å	13	color	142	COD
Bunch et al. (1961)	secondary effluent	cellulose dialysis membrane	27.4	COD	40.3	COD
Gjessing (1971)	surface water	cellophane membranes 0 40Å	65	TOC		
Martin et al. (1972)	fungal humic acid	dialysis membrane	97%	weight	3600	weight
	fungal fulvic acid		29%	weight	1400	weight
Packham (1964)	organics from surface water concentrated by ion exchange: humic acid	dialysis membrane 0 24Å	82	weight		
	humic acid	membrane filter 0 100Å	7	weight		
	fulvic acid	dialysis membrane 0 24Å	33	weight		
Stephens (1964)	butanol extractable organics	cellulose dialysis membrane	~20-25	weight		

ULTRAFILTRATION MEMBRANES

New types of highly permeable ultrafiltration (UF) membranes consisting of anisotropic polyelectrolyte complexes (Amicon, Lexington, Mass) and cellulose acetates (Abcor, Cambridge, Mass) have become available since 1960. Isotropic microporous cellulose ester membranes, such as Pellicon molecular filters (Millipore, Bedford, Mass.) and Nuclepore membranes (Nucleopore Pleasanton, CA), manufactured by etching of radiation damaged sites, have become available only recently (Good, 1974). These membranes have a sharper molecular cutoff than most ultrafilters owing to their isoporous structure. Cellulose acetate ultrafilter are also commercially available (Biorad, Richmond, CA).

Several studies have used membrane ultrafiltration to characterize molecular sizes of aquatic organics and their experimental procedures are listed in Table 8. When more than one ultrafiltration membrane is used, a parallel procedure can be selected in which the sample is divided in different aliquots that are each passed through the individual membrane (Gjessing, 1975; Gjessing, 1970). In the sequential procedure, the sample is passed either through the membrane with the largest pore size whereupon the permeate is passed through the membrane with the next smaller pore size (Calder and Kearsley, 1974) or it is first passed through the membrane with the smallest pore size whereupon the retentate is passed through the membrane with the next largest pore size (Doig and Martin, 1974; Gjessing, 1970). None of the studies, however, evaluated the difference among the three procedures. After comparing a dialysis membrane with the ultrafiltration membranes UM-10 and XM-50, Gjessing (1971) noted that 66% of the organic carbon was retained by a cellophane membrane with a stated pore size of 40 Å and a reported molecular-weight of 10,000; the ultrafiltration membranes with molecular-weight cutoffs of 10,000 and 50,000 retained 94% and 50%,respectively, on a comparable sample. Interpolation of the rejection values would indicate that 66% of the organic carbon would be retained with a UF membrane with a molecular-weight cutoff of 30,000 which is about three times larger than the reported molecular-weight

Table 8. Membrane Ultrafiltration Studies Characterizing the Molecular Weight Distribution of Aquatic Organic

Author	Ultrafiltration Membranes	Source	Pretreatment	Organic carbon content (mg/l)	Filtration Steps	Washing of retentate	Concentration Factor
Barber (1968)	UM3	Atlantic Ocean	no	0.6-1.9		no	two 7x
Calder and Heartley (1974)	UM2, PM10, XM50, XM100A	marsh water	glass fiber filter	6.2-8.5	sequential high MW-low MW	yes	
		salty marsh water	glass fiber filter	3.8-5.4	sequential high MW-low MW	yes	
Cameron et al. (1972)	XM50	soil humic acid	pyrophosphate extract				
Daly and Martin (1974)	UM05, UM2, UM10	3 water extract of 250g soil	0.22	67	sequential low MW-high MW	yes	
Shapiro (1967)	UM1	vacuum concentrated surface water	paper filter			no	10x
Gjessing (1971)	UM10, XM50	4x concentrated surface water	glass fiber filter	15.2	parallel		
Gjessing (1973)	UM2, UM10, XM100	surface water	glass fiber filter	12.5	parallel	yes	40x
Gjessing (1973)	UM2, UM10, XM300	10x concentrated surface water	glass fiber filter	15-28	sequential low MW-high MW	yes till permeate <5 mg/l TOC	
Schindler and Alberts (1974)	UM2, UM10, XM50, XM100	lake water	glass fiber	1.2-7.8	parallel		
Allender (1972)	UM2, PM10	surface water		10-300	parallel	no	10x
Zajic and Moffitt (1975)	UM10, XM50	hydrocarbon solution	0.45μ, dialysis with 400 cellulose membrane		sequential low MW-high MW	yes	

cutoff of the dialysis membrane. Similarly, Ferry (1936) noted that while iso-electric serum albumin would not penetrate a collodion ultrafiltration membrane, it was still able to dialyze through a collodion membrane with a pore diameter one third of the UF membrane. Elford (1933) also observed that substances that would pass collodion membranes during dialysis will be retained during ultrafiltration using the same membrane as a result of partial blocking of the pores.

COMPARISON OF MEMBRANE SEPARATION METHODS WITH OTHER SEPARATION TECHNIQUES

Only a few studies confirmed the obtained molecular-weight distribution of aquatic organics by independent methods. Sieburth and Jensen (1969) noted that organics included in Sephadex G-15, thereby having a molecular smaller than 1500, all permeated through a dialysis membrane, while DeHaan (1974) noted that organics included in G-25, thereby having a molecular weight smaller than 5000, passed through a dialysis membrane. Gjessing (1973) further showed that membrane fractionation using ultrafiltration membranes gave better separation than gel permeation chromatography. The organics excluded from Sephadex G-75, thereby having a molecular weight larger than 60,000, for example, still permeated partially (19%) through a 10,000 MW UF membrane, while the organics included in the G-10, thereby having a molecular weight smaller than 500, were partially retained (29%) by a 100,000 MW UF membrane. The combined use of membrane ultrafiltration followed by gel permeation of the UF retentate, however, gave the best separation. A quantitative comparison of the two separation processes was not made, since the UF was monitored by TOC and the Sephadex by absorbance (Gjessing, 1973).

It was therefore the purpose of the present study to quantitatively compare membrane ultrafiltration and gelpermeation chromatography. The present study also evaluated the parallel versus the sequential UF separation procedures. Although, most of the previous studies have tested water with a low organic matter content (Table 8), the present study evaluated the applicability of UF separation using high strength leachate. The leachate samples having chemical oxygen demand (COD) values ranging from 81 to 71,680 mg/l were obtained from solid waste fills of different ages and subject to different climatic conditions (Chian and DeWalle, 1976). Such leachate is generated by infiltrating rainwater which leaches pollutants from the solid waste. An in-depth knowledge of the organic composition is necessary to assess the potential for groundwater pollution and to predict the effectiveness of treatment processes to remove pollutants from the leachate. A summary of previous studies (Table 9) showed similar ranges of organic matter concentration. It was also noted that the major fraction consisted of free volatile fatty acids which was also reflected in the high biochemical oxygen demand (BOD) relative to the chemical oxygen demand. The present study also included the constituents listed in Table 9 but used membrane ultrafiltration, gel permeation chromatography and specific organic analyses to account for the unidentified major organic fractions.

Table 9. Organic Matter Concentrations in Landfill Leachate and their Relative Composition

Author	Organic Matter concentration	Relative organic matter composition
Burrows and Row (1975)	170,000 mg/l COD	79% of COD as fatty acids 4% of COD as alcohols
County of Sonoma (1973)	3260-22,400 mg/l COD	40% of COD as fatty acids 80% of COD as BOD
Hughes *et al.* (1971)	1050-50,000 mg/l COD	25% of COD as fatty acids
Lin (1966)	20,000 mg/l COD	75% of anions from ionized fatty acids
Mao and Pohland (1973)	4320-12,000 mg/l COD	60% of COD as fatty acids 74% of COD as BOD
Qasim and Burchinal (1970)	33,360 mg/l BOD	35% of organic matter as aromatic hydroxyls 32% of organic matter as nitrogeneous organics

MATERIALS AND METHODS

The ultrafiltration membranes used in this study were UM05, UM2, UM10, and XM100A (Amicon, Lexington, MA) with nominal molecular-weight cutoffs of 500, 1000, 10,000 and 100,000, respectively. After ultracentrifugation of one liter of leachate at 30,000 rpm for 30 minutes, the supernatant was concentrated 5 fold with a 500 MW UF membrane. The retentate was then desalted by diluting to twice its volume with distilled water followed by a reduction to the original retentate volume; this step was repeated a second time. The percentage retained was calculated from a material balance. The 500 MW UF membrane was selected since it has the lowest molecular-weight cutoff that is able to separate the organic matter from the inorganic salts that are present in high concentration in the leachate.

Following the ultrafiltration step, separate aliquots of the UF retentate were further separated into different molecular-weight components using Sephadex G-75 (1000-50,000 MW) and G-200 (1000-200,000 MW) columns (Pharmacia, Piscataway, NJ). After the molecular weight distribution of the 500 MW UF retentate was established by the use of the Sephadex columns, other ultrafiltration membranes with higher molecular weight cutoffs were employed to obtain sufficient quantities of each of the different molecular weight fractions for chemical analyses.

The ultrafiltration membrane fractions and Sephadex fractions were characterized by TOC (TOC Analyzer 915A, Beckman, Fullerton, CA), COD, carbohydrates as dextrose (anthrone test), amino acids as lysine (ninhydrin test after 24 h. acid digestion with 6N HCl), carboxyl groups as acetic acid (hydroxyl amine test), carbonyl groups as acetophenone (2, 4-dinitrophenylhydrazine test), and aromatic hydroxyl groups as tannic acid (Denis test). Dissolved free amino acids were measured with an automated Technicon TSI amino acid analyzer (Tarrytown, NY).

Each membrane fraction was also separated according to its polarity by two solvent extractions: hexane, which separates the less polar lipids, hydrocarbons and fatty acids from the water phase, and butanol, after pH adjustment of the aqueous layer to 2, which separates humic substances from the water phase (Rebhun and Manka, 1971; Packham, 1964; Christman and Ghassemi, 1966). The hexane extraction was performed twice on a 20-ml sample of the concentrated leachate with 2 portions of 20-ml hexane heated to 45°C, which after vigorous shaking formed an emulsion. This was eliminated by addition of 5 ml ethanol and 0.5 ml of 1N H_2SO_4, after which the hexane layer was removed from the separatory funnel. After acidification of the aqueous layer to pH 2, it was further extracted twice with two 20-ml aliquots of butanol. An additional 3.5 ml of ethanol was added to break the emulsion. After separation of the solvent layers from the water phase, the solvents were removed.

Infrared (IR) spectra were run on the residues of the hexane and butanol extracts using a Beckman Model 20A (Fullerton, CA). Gas chromatographic (GC) analyses of the free volatile fatty acids were conducted using a Hewlett Packard 5750 B Chromatograph (Palo Alto, CA) equipped with a flame ionization detector and a column consisting of 20% neopentyl glycol succinate and 2% phosphoric acid on Chromosorb PAW 60-80 mesh (Chian and Mateles, 1969).

The most extensive organic analysis was conducted on a leachate sample obtained from a large experimental lysimeter installed at the University of Illinois. The lysimeter - 1.51 m in diameter and 3.02 m in height - was filled with 1525 kg of milled solid waste (obtained from the Hartwell section of Cincinnati, Ohio) which consisted of 44% paper and 12% food waste. Based on the analysis of this leachate sample the most important parameters were selected and subsequently measured on leachate samples collected from selected landfills in different locations of the United States.

RESULTS

It was observed that the color of the leachate sample collected from the underdrain of the lysimeter changed rapidly from light yellow to dark green, and that after prolonged storage a dark brown iron hydroxide precipitate accumulated on the bottom of the sample container. The sample, with a TOC of 17,060 mg/l and a COD of 49,300 mg/l, has a corresponding iron concentration of 2200 mg/l. Since the colloidal iron hydroxide would interfere with the membrane separation processes, the leachate sample was collected under anaerobic conditions. To prevent further oxidation of the iron, the sample was processed as rapidly as possible through the ultracentrifugation and membrane ultrafiltration step under nitrogen atmosphere.

SEPARATION BY MEMBRANE ULTRAFILTRATION AND GEL PERMEATION

Only 104 mg/l or 0.6% of the volatile residue of 18,403 mg/l was removed with the ultracentrifuge indicating that most organics are present in the soluble fraction. Subsequent membrane ultrafiltration showed that 27.2% of the initial TOC of the sample was retained by the 500 MW UF membrane: thus, because the majority passed the membrane, they may be identified as low-molecular-weight compounds. When the 500 MW UF retentate was applied on a Sephadex G-75 column, the TOC distribution in the eluate showed that 22% of the 500 MW UF retentate or 6% of the original TOC was excluded from the column as it eluted between 20 and 36 ml (Figure 6). This exclusion indicates a molecular weight larger than approximately 30,000-50,000 MW. The application of the 500 MW UF retentate to a Sephadex G-200 column resulted in essentially the same elution pattern. This fraction, therefore, had a molecular weight considerably larger than 50,000 (Fisher, 1965). The results in Figure 6 further showed that the majority of the 500 MW UF retentate consisted of organics with a molecular weight smaller than 1000 to 3000 (Table 10).

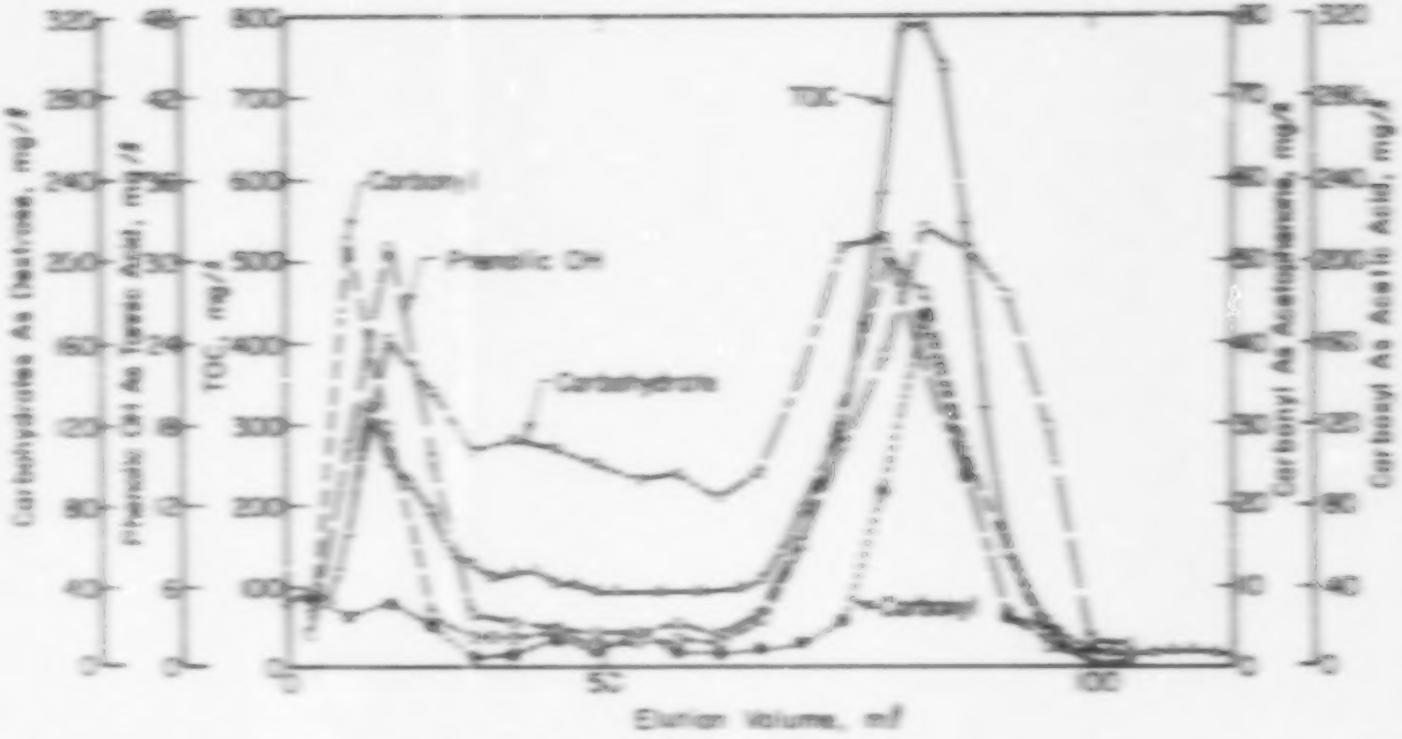

Figure 6. Eluate of the 500 MW UF Retentate of the OI Leachate Sample on a Sephadex G-75 Column as Characterized by Total Organic Carbon, Specific Organics and Functional Groups.

Table 10. Chemical Analyses of the Sephadex G-75 Eluate of the 500 MW UF Retentate

	High MW Fraction (1a)	Intermediate MW Fraction (2a)	Low MW Fraction (3a)
Estimated MW	~(30,000-50,000)	~3000-30,000	~(1000-3000)
Elution volume	20-36 ml	36-66 ml	65-100 ml
%TOC of 500 MW UF retentate	22%	10%	68%
%TOC of original sample	6%	2.5%	18.7%
% of organic carbon consisting of carbohydrates	26%	42%	7%
% of organic carbon consisting of aromatic hydroxyl compounds	4%	2%	6%
% of organic carbon consisting of carbonyl compounds	1%	2%	5%
% of organic carbon consisting of carboxyl compounds	5%	5%	6%

Substantial quantities of the different molecular weight fractions were obtained using UF membranes having larger molecular weight cutoffs than the previously used 500 MW UF membrane. Based on the gel permeation chromatographic data, both a 10,000 MW and a 100,000 MW UF membrane were selected. When another aliquot of the centrifuged leachate was passed through a 10,000 MW UF membrane, 9.9% of the TOC of the sample was recovered in the retentate (Fraction 3b, Figure 7). The results indicate that the 10,000 MW UF membrane not only retained the Sephadex fractions 1a and 2a in (Table 9) but also part of the low-molecular-weight Sephadex fraction 3a. As 9.9% of the initial TOC would represent organics eluted from the G-75 up to 69 ml (Figure 6) corresponding to an apparent molecular weight of 2500, it would indicate that the nominal molecular weight estimated from the membrane ultrafiltration is approximately four times higher than the absolute molecular weight obtained from the gel permeation. When the 10,000 MW UF permeate was subsequently passed through a 500 MW membrane, the sum of the TOC of the 10,000 and 500 MW retentate (Fractions 3b and 4b, Figure 7) was only 19.6% of the original TOC of the sample, as compared to 27.2% when an aliquot was passed through the 500 MW UF directly (fraction 6b, Figure 7). Since the 10,000 MW UF permeate when applied to the 500 MW UF was devoid of large quantities of high molecular weight compounds and ferric hydroxide colloids, the absence of these compounds appeared to decrease the rejection with the 500 MW UF.

To further investigate this apparent decrease in rejection, an aliquot of the original 500 MW UF retentate was diluted to its original strength and pH and passed through the 10,000 MW UF membrane. The retentate (Fraction 8b, Figure 7) was found to represent 21.8% of the original TOC as compared to 9.9% when the centrifuged leachate directly permeated the 10,000 MW UF membrane. This indicates that a relatively large concentration of high-molecular-weight organics together with ferric hydroxides will decrease the nominal molecular-weight cutoff of the membrane. This was further exemplified by the application of fraction 8b to a 100,000 MW UF

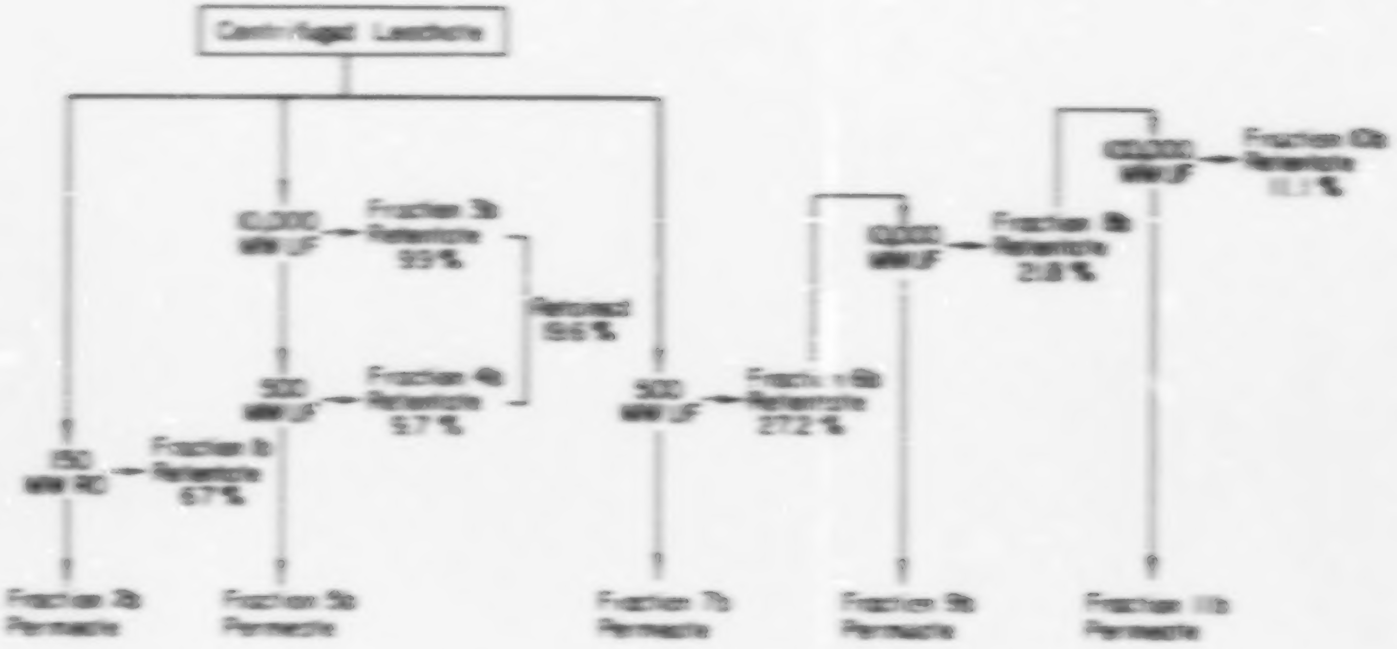

Figure 7. Effect of Parallel and Sequential Membrane Separation on the Retention of Organic Carbon in Centrifuged Leachate by Reverse Osmosis and Ultrafiltration Membranes having Different Molecular-Weight Cutoffs.

membrane after dilution to its original strength. The 100,000 MW UF membrane was found to retain 11.1% of the original TOC (Fraction 10b, Figure 7) which is larger than the 9.9% (Fraction 3b, Figure 7) obtained when the sample directly permeated the 10,000 MW UF membrane. Thus, the sequence of membrane ultrafiltration steps may change the nominal molecular-weight cutoff of a membrane by a factor of more than 10. When the 500 MW UF retentate permeated the membranes with the next highest molecular-weight cutoff, most of the salts were already removed and present in the 500 MW UF permeate. The reduced salt content may result in an increase of the diffuse double layer and an increase of the size of the molecule (Gjessing, 1971), which in turn will increase its rejection. The above results indicate that parallel ultrafiltration will result in a more representative separation than serial ultrafiltration. Because the 500 and 10,000 MW UF membranes are best suited to separate the organics in leachate, they were selected and used in the parallel procedure to separate the organics in other leachate samples.

SPECIFIC ORGANIC ANALYSIS OF DIFFERENT MOLECULAR-WEIGHT FRACTIONS

Extensive organic analyses were conducted on the organic fractions obtained from the gelpermeation and membrane ultrafiltration steps. Analyses of the specific organics present in the Sephadex fractions showed considerable differences (Figure 7; Table 10) with relatively high concentrations of carbohydrates observed in the high-molecular-weight fraction and substantial quantities of aromatic hydroxyl and carboxylic compounds present in the low-molecular-weight fraction. Although the TOC data apparently indicate that fraction 3a is rather homogeneous (Figure 6), the colorimetric tests show that this is not the case since the maxima of the different organic compounds do not elute at the same volume, and the specific compounds associated with organics of decreasing molecular weight are carbohydrates (maximum at 77 ml), carbonyl- (81 ml), carboxyl- (83 ml) and aromatic hydroxyl compounds (85 ml).

Relative constribution to the carbon in each molecular weight fraction was then calculated (Table 10). It was thereby assumed that the carbon in the model compounds used in the colorimetric tests reflected the actual structures and that a certain organic structure did not respond to more than one colorimetric test. A TOC/weight ratio of 0.4 for carbohydrates and carboxyl compounds and a ratio of 0.5 for aromatic hydroxyl and carbonyl compounds were used to calculate the values in Table 10. Although the model compound in the carbonyl test had a TOC/weight ratio larger than 0.5, the value of 0.5 was selected since the carbon generally does not exceed more than half of the weight of humic substances (Kononova, 1966).

Organic analyses of the membrane UF fractions 3b and 10b (Figure 7) showed that 23% and 24% of the organic carbon consisted of carbohydrates which is comparable to the value of 26% obtained in the larger than 50,000 MW Sephadex fraction. Analyses of the other molecular weight fractions (Figure 8) showed that the relative carbohydrate content decreased with decreasing molecular weight, thereby, confirming the results of the Sephadex analyses. These data also show that the carbohydrates and aromatic hydroxyl groups can be selected as indicators for the presence of high-and low-molecular-weight organics (Fraction 10b and 9b, Figure 7) respectively, since the carbohydrates are mainly present in the high-molecular-weight fraction while the aromatic hydroxyls are present in highest concentration in the low-molecular-weight organics.

The protein test was run on the different membrane fractions and its values followed a trend similar to the carbohydrates in the fractions larger than 10,000 MW (Figure 8). A surprisingly high content of amino acids was found in the 500 MW permeate indicating the presence of free amino acids that permeate the 500 MW UF membrane. Qualitative analysis of the unhydrolyzed 500 MW UF permeate with a Technicon automated amino acid analyser showed in decreasing order thepresence of ornithine, lysine and valine. Dissolved free amino acids are generally detected

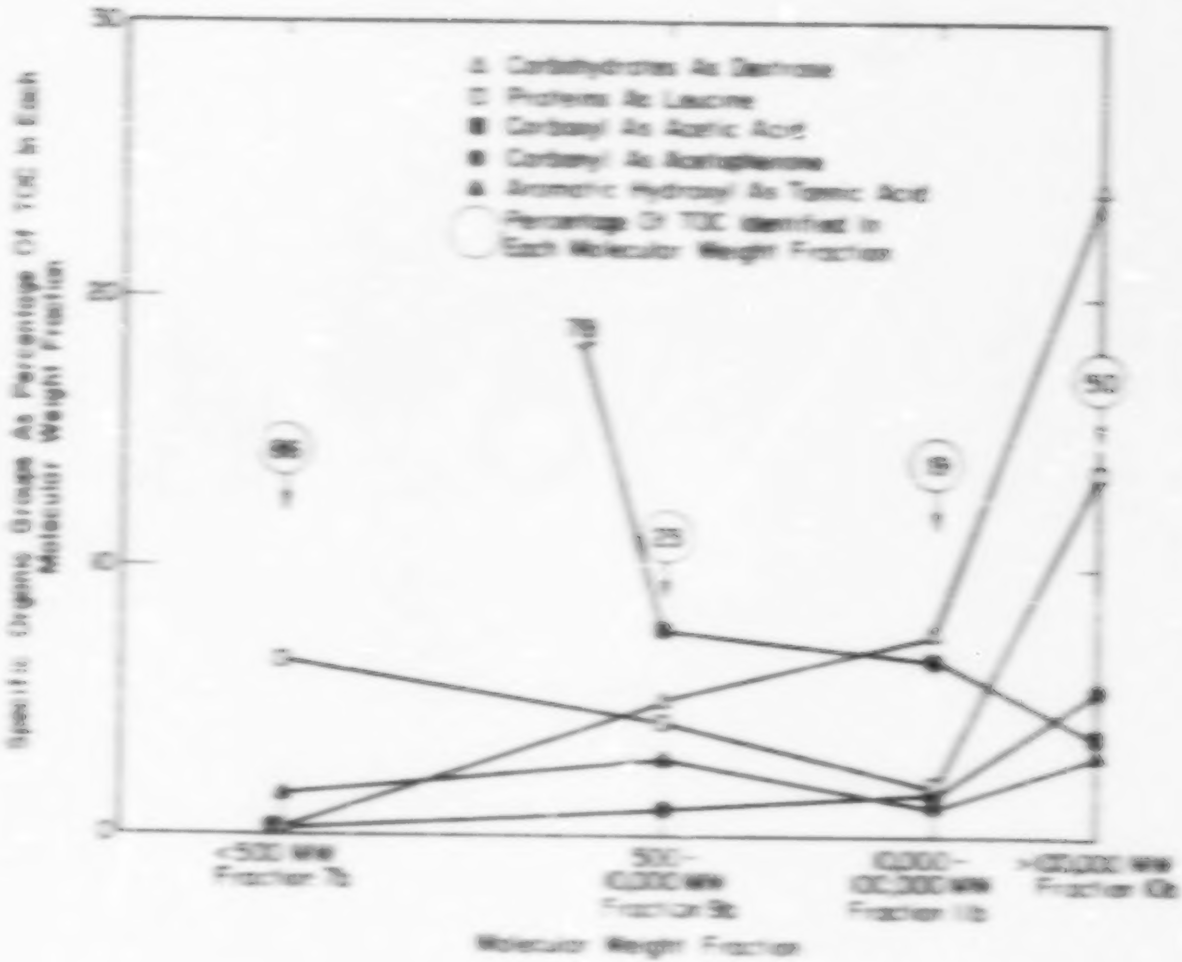

Figure 8. Characterization of Different Membrane Ultrafiltration Fractions for Specific Organics and Functional Groups

when nitrogen is not a limiting nutrient source; and the presence of 248 mg/l NH_4^+-N in the leachate sample indicated that this was probably the case. Comparison of the amino acids measured with ninhydrine method after 6N HCl hydrolysis to the organic nitrogen content measured with Kjeldahl method and multiplied by 6.25 (Bunch _et al._, 1966) showed that 90% of the nitrogeneous compounds were present as free or bound amino acids.

Analyses of the carboxyl compounds showed that the organics associated with this group increased in the membrane fractions having decreasing molecular weight (Figure 8) confirming the results in Figure 6. A titration with NaOH was made using Fraction 8b and 9b (Figure 7) which after acidification with HCl showed a weak inflection point at a pH of 4.30 for fraction 8b and at 4.37 for fraction 9b. A sharper inflection point was noted at 7.2 for both fractions. Assuming that the amount of base necessary to raise the pH of the solution from the first inflection point to the second is due solely to the dissociation of the carboxyl group, it was calculated that the density of carboxyl groups was 10.1 meq/gC for fraction 9b. The corresponding density as measured with the hydroxyl amine test indicated that 8.0% of the carbon of that membrane fraction was associated with the carboxyl group. The titration resulted in a density of 6.6 meq/g C for fraction 8b. The corresponding value measured with the hydroxylamine test was 5.2%, showing that both the titration and hydroxyl amine test resulted in values of the same relative magnitude.

Owing to the presence of the free volatile fatty acids that pass the 500 MW UF membrane, the highest percentage of carboxyl groups was present in the 500 MW permeate. Further analysis of the 500 MW UF permeate by GC showed the presence of acetic acid (1748 mg/l), propionoic acid (509 mg/l), isobutyric acid (312 mg/l), butyric acid (3074 mg/l), isovaleric acid (717 mg/l), valeric acid (564 mg/l), and hexanoic acid (1888 mg/l). The carbon from these free volatile fatty acids comprised 78% of the TOC of the 500 MW UF permeate or 49% of the initial TOC of the leachate sample.

The results of the analyses showed that as much as 86% of the carbon present in the 500 MW UF permeate was identified, followed by 50% identified in the 100,000 MW UF retentate. The intermediate fractions were least characterized indicating that they consisted of complex molecular structures. Of the total organics present in the sample 71% was identified.

SOLVENT EXTRACTION OF DIFFERENT MOLECULAR WEIGHT FRACTIONS

The last step in the characterization scheme consisted of solvent extraction to separate the different membrane UF fractions according to their polarity. The hexane extraction of the 10,000 MW UF retentate (fraction 3b, Figure 7) showed that this step extracted only 13.8 mg/l. If most of the hexane extract consisted of palmitic acid (Shorland, 1963), to give an organic carbon/weight ratio of 0.75, this fraction would represent only 0.5% of the organic carbon in the 10,000 MW UF retentate (Table 11). The residue of the hexane extract had a relatively white to light brown color and a very sharp and pungent odor. Since the hexane residue contained some dark brown iron hydroxide colloids originating from the water layer, the hexane layer was filtered prior to drying.

The IR spectrum of the residue (Figure 9a) showed strong absorption bands near 2900 cm^{-1} and 1470 and 1390 cm^{-1}, indicating the dominant influence of aliphatic structures (Table 12). The band at 1720 cm^{-1} represents the C=O stretch of the carbonyl in the carboxyl group whereas the shoulder at 1750 cm^{-1} reflects the fraction of carbonyls associated with ester linkages which also shows at 1270 cm^{-1} (Table 12). The IR spectrum therefore, would indicate that the majority of this fraction consists indeed of fatty acids some of which are still present in ester linkages. Colorimetric analysis of the redissolved hexane residue showed presence of carbohydrates. These results therefore indicate that the residue of the hexane extract will slightly overestimate the concentration of fatty acids because other organic fragments are also extracted.

Table 11. Effectiveness of the Hexane and Butanol Extraction to Remove Organics from the Different Molecular Weight Fractions

Membrane or Fraction	Hexane extract as percentage of the carbon	Extraction of specific organic	Butanol extract as percentage of the carbon	Extraction of specific organics
10,000 MW UF retentate (Fraction 3b)	1.1	3.3% of carbohydrates in UF retentate extracted with hexane	2.1	4% of carbohydrates and 100% of aromatic hydroxyls present in UF retentate extracted with butanol
500 MW UF retentate combined 10,000 MW and 100,000 MW UF permeate (Fraction 3b and 3c)	4		8	100% of aromatic hydroxyls present in UF retentate extracted with butanol
500 MW UF permeate (Fraction 3c)	1.5	3.5% of carbohydrates 70% of aromatic hydroxyls present in UF permeate extracted with hexane	7	45% of aromatic hydroxyls present in UF permeate extracted with butanol

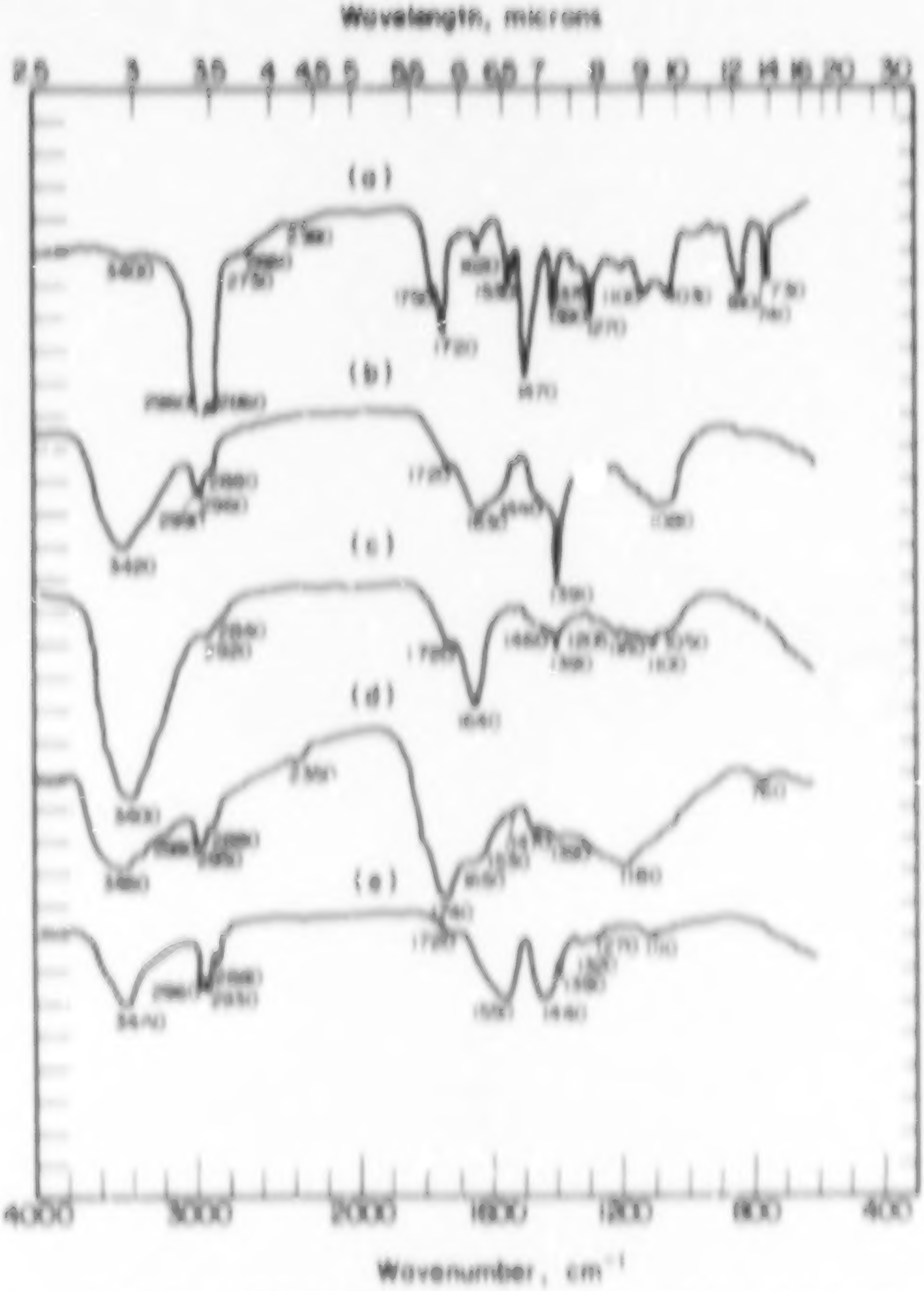

Figure 9. IR Spectra of the Residue of the Hexane and Butanol Extracts: (a) Hexane Extract of the 10,000 MW UF Retentate (b) Butanol Extract of the 10,000 MW UF Retentate (c) Butanol Extract of the hydrolysed 10,000 MW UF Retentate (d) Butanol Extract of the 500 MW UF Retentate (e) Hexane Extract of the 500 MW UF Permeate

Table 12. Infrared Absorption Bands and their Tentative Assignments for the Residue of the Hexane and Butanol Extracts

Hexane extract of 10,000 MW UF retentate (Fraction 3b)	Butanol extract of 10,000 MW UF retentate (Fraction 3b)	Acidified butanol extract of 10,000 MW UF RETENTATE (Fraction 3b)	Butanol extract of 500 MW UF retentate (Fraction 9b and 11b)	Hexane extract of 500 MW UF permeate (Fraction 7b)	
3400	3420	3400	3460	3440	H bonded O-H stretch, N-H stretch
2960	2990		2950	2960	methyl C-H stretch
	2960	2920	2950	2930	methylene C-H stretch
2820	2860	2840	2860	2860	aldehyde C-H stretch
2730					carboxyl O-H stretch
2380			2350		phosphorus P-OH stretch
1750			1760		carbonyl C=O stretch in esters
1720	1720	1720		1720	carbonyl C=O stretch in carboxyl groups
	1630	1640	1650		N-H bending, C=C stretch, C=O stretch of ketones, amides and carboxylate ions
1620					N-H bending
1530				1550	N-H bending, C=N stretch of amides
1470	1460	1460	1470	1460	CH_2 scissor bending; benzene skeletal vibration
1380	1380	1390	1390	1380	Symmetric C-CH_3 bending; O-H bending of carboxylic acid
					Symmetric O-C-O stretch of carboxylate ion
1270					Symmetric C-O-C stretch of esters C-OH stretch of carboxylic acid
		1160	1180		aromatic structures
1100		1100		1100	C-O stretch of cyclic alcohol aromatic structures
1030	1080	1050			C-N stretch of amino groups
810					C-O stretch of cyclic alcohol aromatic structures
			782		out of plane bending of NH_2
760					aromatic out of plane bending of C-H
730					CH_2 rocking vibrations

The residue of the butanol extraction of the 10,000 MW UF retentate was of a greater magnitude than that of the hexane extract. If 50% of the weight of the butanol residue consisted of carbon, 2.5% of the organic carbon in the 10,000 MW UF retentate is comprised of this group (Table 11). The residue of the butanol extract had a dark brown oily appearance and an acidic and 'moldy' odor. The IR spectrum of the butanol residue of the 10,000 MW UF retentate of the lysimeter sample (Figure 9b) showed the dominant influence of hydroxyl groups such as those present in carbohydrates (1080 cm^{-1}) and carbonyl groups (1640 cm^{-1}). The strong peak at 1390 cm^{-1} is probably due to the scissor bending of a CH_2 group adjacent to a carbonyl group. The IR spectrum is almost identical to a humic - carbohydrate-like high molecular weight fraction isolated from effluent from an activated sludge unit (DeWalle and Chian, 1974). Further acidification of the dry residue with HCl and subsequent drying reduced the peak at 1390 cm^{-1}. This reduction indicates that part of the peak was contributed by the symmetric O-C-O stretch of carboxylate ions. Since the carbonyl stretch at 1630 cm^{-1} did not shift to 1720 cm^{-1}, however, the majority of the carbonyl groups in this fraction are present not as carboxyl carbonyls, but as aldehydes and ketones. Moreover, the reduction of the peak at 1080 cm^{-1} may indicate that some carbohydrates were hydrolyzed (Tan, 1970).

Colorimetric tests confirmed the presence of carbohydrates and aromatic compounds. While only 8% of the carbohydrates present in the 10,000 MW UF retentate were removed, all of the aromatic hydroxyl groups were extracted with the butanol (Table 11). If the model compound reflects the actual extracted organics, it can be calculated that about 30% of the weight of the butanol extract of the 10,000 MW UF retentate was associated with aromatic hydroxyl compounds. Since the aromatic compounds only represent about 3% of the carbon in this fraction, it was concluded that the butanol preferentially extracted this group. The above results of the extraction were confirmed by using model compounds that resemble the different classes of organics present in the 10,000 MW UF retentate. The removal of 93% by butanol extraction of a 1000 mg/l tannic acid solution demonstrated the affinity of butanol towards aromatic hydroxyl compounds. Only 14% of a bovine albumin solution was extracted while no removal was observed with a starch solution.

Extraction of the 500 MW UF retentate, i.e., fractions 9b and 11b combined (Figure 7), with hexane did not result in a residue. This indicates that the fatty acids or other non-polar organics are only present in the high molecular weight fraction, a conclusion similar to the one reached by Ogner and Schnitzer (1970). The butanol extract, however, was substantially larger and represented 9% of the TOC (Table 11). The butanol layer was not as dark brown as that of the 10,000 MW retentate (Fraction 3b, Figure 7). Before complete evaporation of the butanol a white semi-crystalline substance was visible which darkened after the last butanol had evaporated. The appearance in the IR spectrum (Figure 9) of strong influences of the carbonyl in the carboxyl group at 1740 cm^{-1} (Table 12) indicated that the carboxyl group was more dominant in this fraction than in the butanol extract of the 10,000 MW UF retentate. This trend parallels the results of the hydroxylamine test for carboxyls (Figure 8). Comparison with other IR spectra (Black and Christman, 1963; Stevenson and Goh, 1971) showed that this fraction resembled a fulvic acid. The butanol was able to extract almost 100% of the aromatic hydroxyls present in this fraction (Table 11).

The hexane extract of the 500 MW permeate represented 1.5% of the organic carbon of that fraction. The IR spectrum (Figure 9) showed two dominant peaks at 1550 and 1440 cm^{-1} representing the asymmetric and symmetric O-C-O stretch of the ionized carboxyl group. These peaks indicate that the hexane extracted some of the free volatile fatty acids present in the 500 MW UF permeate. The small peak at 1110 cm^{-1} and the broad peak at 3440 cm^{-1} may indicate that some carbohydrates and hydroxyl compounds were also extracted with the hexane, a possibility supported by colorimetric tests (Table 11).

The butanol extract of the 500 MW UF permeate represented 7% of the carbon of that fraction. The IR spectrum was similar to that of the hexane extract of the 500 MW UF retentate with the exception that the peak near 2930 cm^{-1} was smaller, probably because of the extraction of lower molecular weight free volatile fatty acids, while the peak near 3440 cm^{-1} was broader.

The above observations indicate a short-coming of the extraction scheme using hexane and butanol in that the hexane extraction may overestimate the amount of lipids since some organic fragments are also extracted. In addition the hexane will extract some low molecular weight free volatile fatty acids when present in the sample. The butanol used to extract the fulvic acid fraction may also extract some of the free volatile fatty acids. Lamar and Goerlitz (1966) found that although humic substances were the main fraction obtained with butanol extraction at acidic pH, up to 10% of the residue consisted of fatty acids. On the other hand, the butanol does not extract all of the fulvic-like material in the 500 MW UF retentate. DeHaan (1972) similarly noted that only 50% of the color was extracted with butanol at pH 2. Butanol, however, does have a preference for aromatic hydroxyl compounds present in this fraction. DeHaan (1972) noted that butanol preferentially extracts organics with a high carboxyl and carbonyl content.

FRACTIONATION AND ANALYSES OF OTHER SAMPLES

The effectiveness of the membrane fractionation was also tested on two other leachate samples obtained from a control and recirculation pilot landfill of intermediate age, operated since October 1971 by the City of Santa Rosa (CA) (County of Sonoma, 1973). The membrane separations were conducted with two aliquots passed separately through either the 500 MW or 10,000 MW UF membrane. The 10,000 MW UF membrane was found to retain 13% and 12% respectively, whereas the 500 MW UF retained 30% and 22% respectively of the initial TOC of the sample. The results of the specific organics present in each fraction as shown in Figure 10 show a similar trend as observed in Figure 8. Comparison shows that

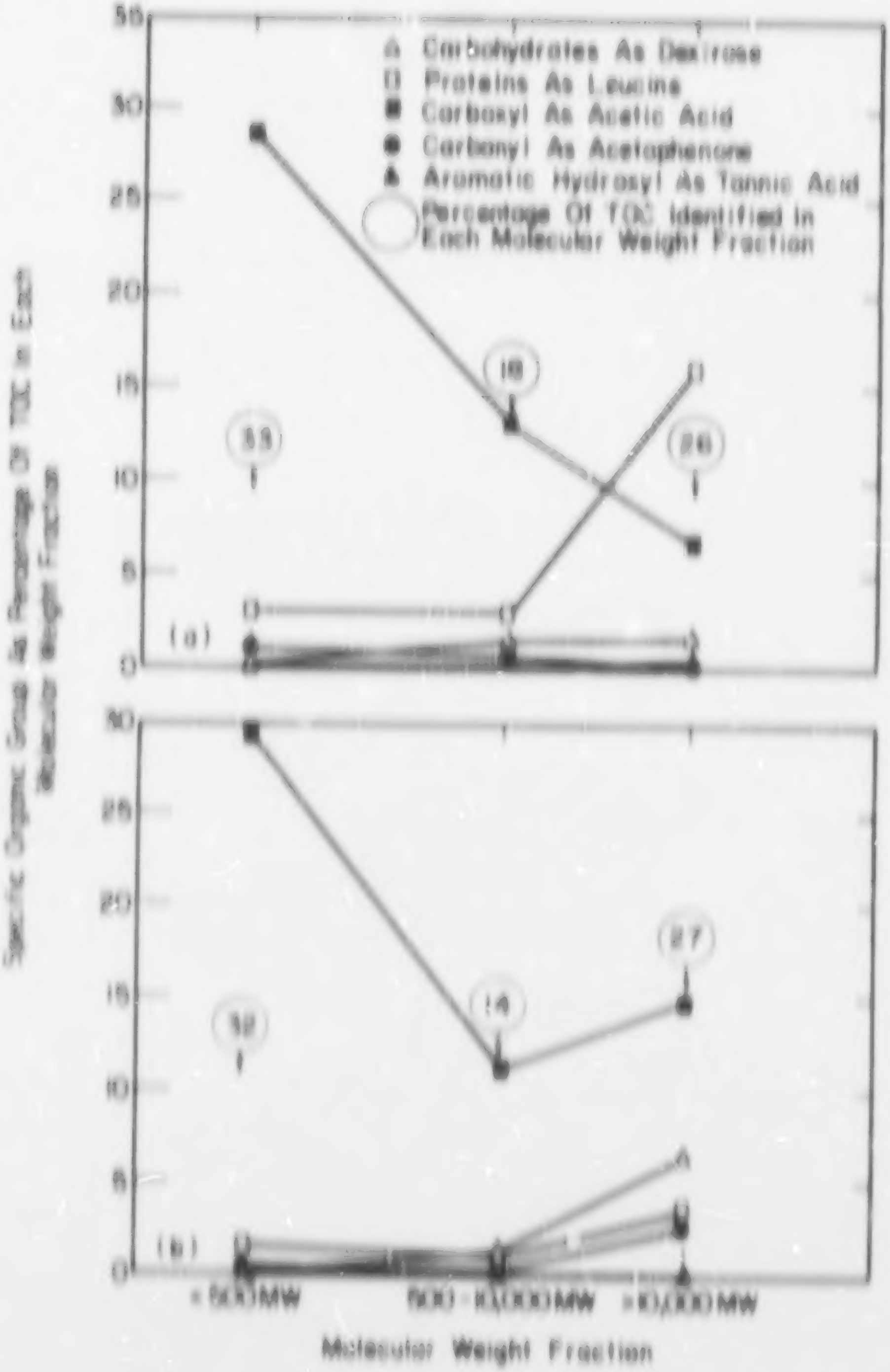

Figure 10. Characterization of Different Membrane UF Fractions in a Leachate Sample Collected from a Control (a) and a Recirculation (b) Landfill in Sonoma County

the identified organics represent a lower percentage of the organic carbon. Since the landfill is located in a warmer climate, a relatively larger percentage of the carbohydrates and proteins may have been degraded at the higher temperature. The carboxyl group, on the other hand, is present in relatively large amounts. As it represents one of the most oxidized forms of organic matter, a higher carboxyl group density would indicate a higher degree of oxidation. The greater stabilization of the Sonoma samples is further substantiated by the lower BOD/COD ratio of 0.62 and 0.65 as compared to 0.80 for the University of Illinois sample.

The membrane fractionation was finally tested with a sample obtained from a relatively old landfill in Dupage County in Northern Illinois (Hughes et al., 1971). Concentration through a 16,000 MW UF membrane did not result in retention of any material, while concentration of a separate aliquot through a 500 MW UF membrane only retained 5% of the original TOC of the sample. As the fatty acids were present in this sample in a concentration below that of the detection limit of the GC, it was concluded that most of the organics were present as low molecular weight refractory molecules. The 500 MW UF retentate was further separated using a Sephadex G-75 column (Figure 11). The TOC data show that only 7% of the 500 MW UF retentate corresponding to 0.5% of the original TOC is present as the high molecular weight humic carbohydrates. The percentage of carbohydrates in this MW fraction is approximately equal to the percentage in the high molecular weight fraction of the leachate obtained from younger fills. The concentration of carbohydrates therefore, tends to reflect the magnitude of the high molecular weight fraction independent of the age of the sample. Since the phenolic hydroxyl group obtained relatively high concentrations in the low molecular weight fraction of the 500 MW UF retentate, it may be used as an indicator for the fulvic like material.

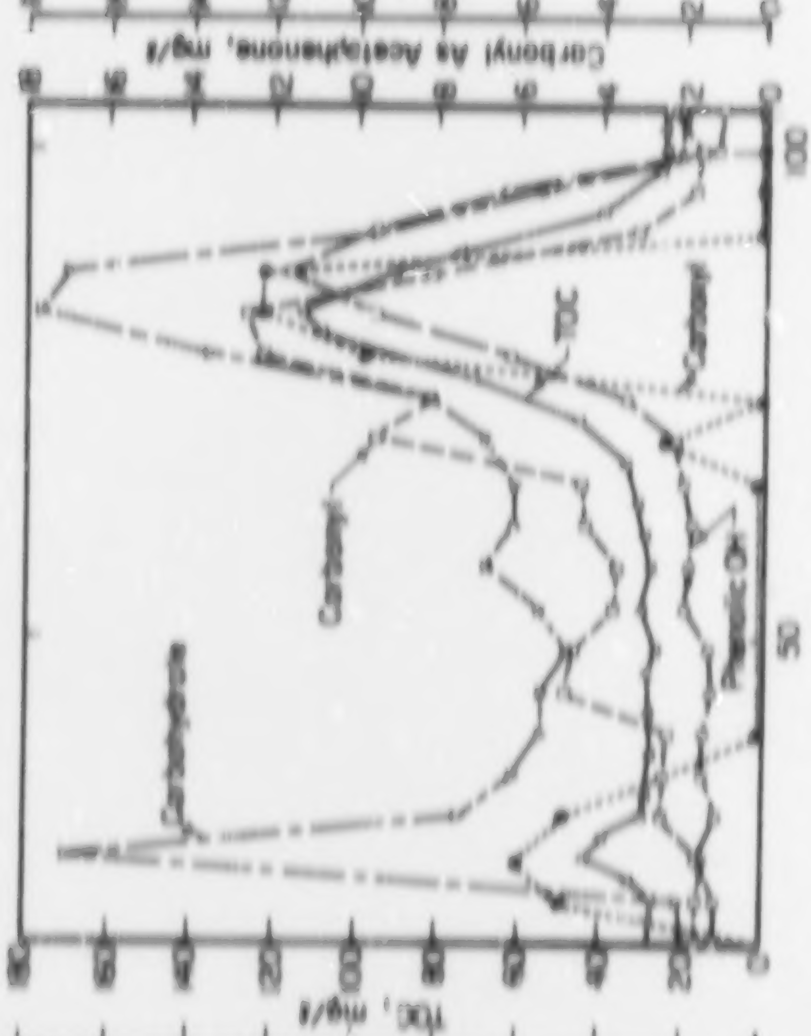

Figure 11. Eluate of the 500 MW UF Retentate of the Dupage County Leachate Sample on a Sephadex G-75 Column as Characterized by Total Organic Carbon Specific Organics and Functional Groups.

The last part of the study consisted of analyzing seven leachate samples in addition to the four that were already studied in greater detail. The samples were collected from different landfills in the United States representing a range of climatic conditions. When the free volatile fatty acids, calculated as percentage of the TOC of the sample, were related to the age of the landfill, the results showed a gradual decrease of the magnitude of this fraction with increasing age of the fill. Since these organics are relatively biodegradable, a similar decrease was observed in the BOD/COD ratios. Separate aliquots were passed through the 10,000 MW UF and the 500 MW UF during membrane separation of the seven samples. However, since some of these samples resulted in a substantial flux decrease, a 1000 MW instead of the 500 MW UF membrane was used. The results of the separation, shown in Figure 12a and 12b, illustrate the gradual decrease of both the high molecular weight humic-carbohydrate-like complex and the lower molecular weight fulvic-like material with increasing age. It was noted that the high molecular weight fraction as obtained in the 10,000 MW UF retentate showed a more rapid decrease then the low molecular weight fraction as obtained in the 500 MW UF retentate. This pattern may indicate that the former fraction is more liable during progressing biodegradation.

Results of the membrane fractionation reflect the changes observed for carbohydrates, proteins and aromatic compounds as measured in the unfractioned leachate samples (Figure 13). The carbohydrates and proteins present in relatively large quantities in the high molecular weight fraction show a relatively more rapid decrease with increasing age of the fill than the aromatic hydroxyl compounds mainly present in the low molecular fulvic-like fraction. These data may therefore indicate that increased stability with respect to biodegradation is observed for free volatile fatty acids < carbohydrates, proteins, humic-like substances < aromatic hydroxyl, carboxyl compounds, and fulvic-like substances.

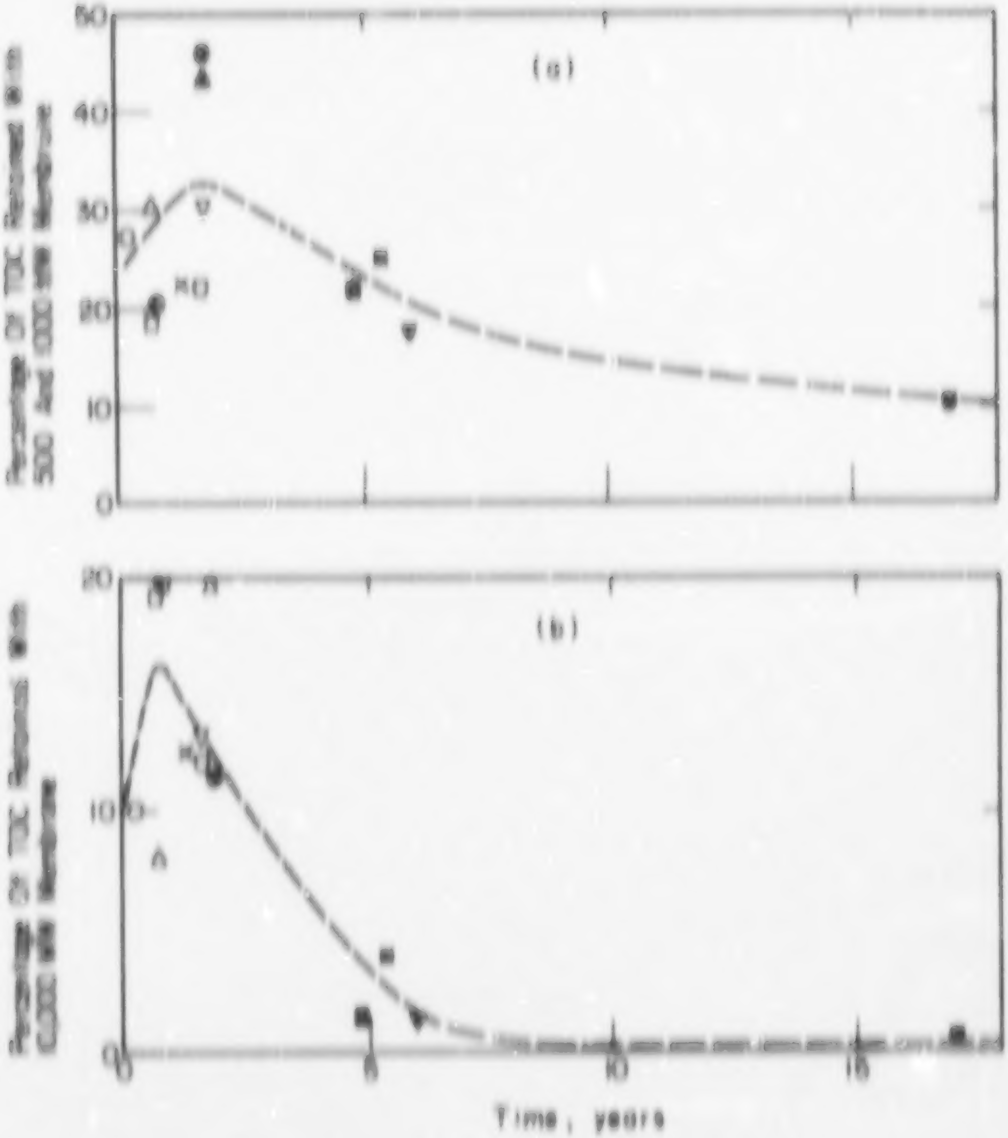

Figure 12. Percentage of TOC Retained with Either 10,000 MW (a) or 500 MW and 1000 MW (b) UF Membranes in Leachate Samples Collected from Landfills of Different Ages

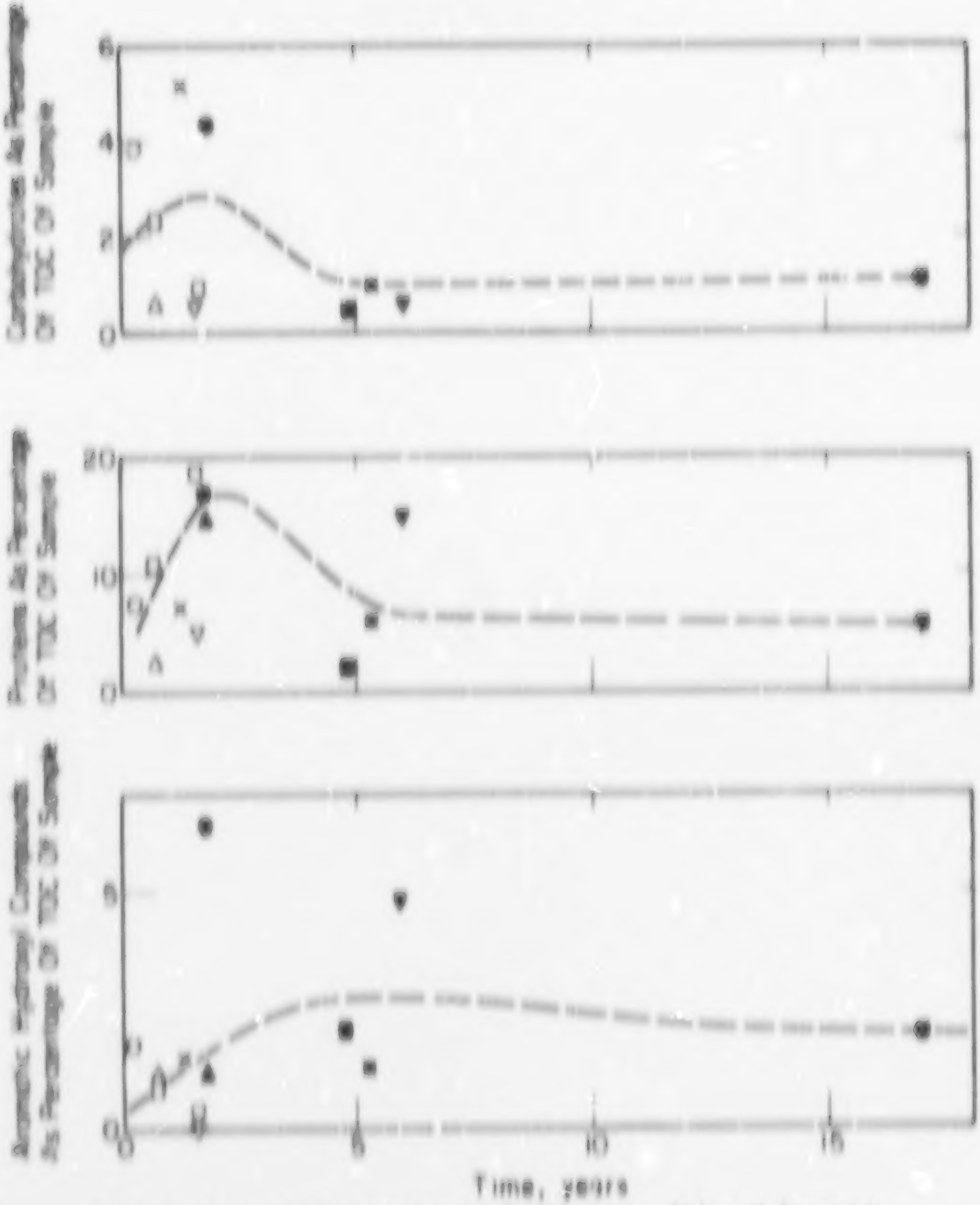

Figure 13. Carbonhydrates (a), Proteins (b) and Aromatic Hydroxyls (c) in Leachate Samples Collected from Landfills of Different Ages.

DISCUSSION

MEMBRANE SEPARATION SEQUENCE

The results of the separation of organic matter by membrane ultrafiltration indicate that a larger percentage of the TOC was retained when the sample was directly passed through the 500 MW UF membrane as compared to the sequential membrane separation through the 10,000 MW followed by the 500 MW UF membrane. Blatt _et al_. (1973) similarly indicated that formation of a polarized layer by the highest molecular species resulted in an increased retention of the intermediate molecular weight fraction present in milk whey; the lowest molecular weight fraction, however, passed through the membranes unimpeded. They also noted that although the rejection of human serum albumin (65,000 MW) with a 100,000 MW UF membrane was zero, its rejection increased rapidly when γ-globulin (160,000 MW) was added. The removal of high molecular weight organics with the 10,000 MW UF membrane, therefore, reduced the degree of polarization at the 500 MW UF membrane during permeation of the 10,000 MW UF permeate. In order to give similar degrees of polarization to each of the ultrafiltration membranes, a parallel ultrafiltration procedure would give more representative results. The least reliable results are obtained when the retentate of the lowest MW UF membrane is passed through UF membranes with successively larger cut offs due to the accumulation of high molecular weight organics that experience such a large degree of polarization, and the absence of salts that would otherwise reduce the double layer of the high molecular weight organics.

COMPARISON OF SEPARATION TECHNIQUES

Comparison of the membrane ultrafiltration and the gelpermeation chromatography showed that the molecular weight estimated by the former method was approximately four times higher than by the latter method. However, this difference may be due to formation of a polarized layer which increased the rejection of the membrane. Formation of such a polarized layer may explain why Gjessing (1971) and Ferry (1936) noted that similar rejections were obtained when the UF membrane had a pore size about three times larger than a dialysis membrane. The opposite effect, was also observed with a sample obtained from a stabilized landfill as no organics were present in the 10,000 MW UF retentate while the excluded Sephadex fraction of the 500 MW UF retentate indicated that 0.9% of the initial TOC should have been retained with the 10,000 MW UF. Discrepancies between the separation techniques may also be due to the nature of each process. Baker and Strathman (1970) showed that membrane rejection of globular organics is higher than that of linear molecules of the same molecular weight due to the deformation and stretching of the latter. A linear molecule for example, was found to have the same retention as a protein one tenth its molecular weight. This behavior is exactly opposite to that noted for both gelpermeation chromatography and dialysis, as they are based on diffusion through microporous capillaries. For example, linear molecules are eluted in the same fraction as protein twice their molecular weight (Fischer, 1969). The discrepancies between the results of the gelpermeation and membrane ultrafiltration could indicate that in the sample obtained from a recent fill globular high molecular weight organics are more dominant, while organics from the stabilized fill have more linear organic molecules. However, additional research is necessary before final conclusions can be made.

ORGANICS IN DIFFERENT MOLECULAR WEIGHT FRACTION

The results of the analysis of the organics separated by membrane fractionation and gelpermeation chromatography tend to confirm earlier studies that separated soil or aquatic humic substances. Table 11 shows that the carbohydrates and hydrolyzable amino acids are present in high concentration in the high molecular weight fraction while carboxyl groups, aromatic hydroxyl groups, color and fluorescence are contributed by fractions of lower molecular weight. Most of the above mentioned studies used methods for functional group analysis

Table [illegible]. Organic Composition of Fractionated Humic Substances

Author	Origin	Organic Fraction	Chemical Composition
[illegible] et al. ([illegible])	soil fulvic acid	high MW excluded from G-25 low MW included in G-25	[illegible] nitrogenous organics, [illegible] as carbohydrates [illegible] nitrogenous organics, [illegible] as carbohydrates Specific absorb. increased with decreasing MW
Schnitzer and Skinner ([illegible])	soil fulvic acid	high MW excluded from G-50	[illegible] meq/g ([illegible] meq/gC) carboxyl groups [illegible] meq/g aromatic hydroxyl groups [illegible] nitrogenous organics
		low MW excluded from G-10	[illegible] meq/g ([illegible] meq/gC) carboxyl groups [illegible] meq/g aromatic hydroxyl groups [illegible] nitrogenous organics C=O and C-OH decreased with decreasing MW
Swift and Posner ([illegible])	soil humic acid	different Sephadex grades	[illegible] nitrogenous organics in high MW fractions [illegible] nitrogenous organics in low MW fractions
[illegible] and [illegible] ([illegible])	soil fulvic acid	neutral charged high MW fraction	[illegible] carbohydrates [illegible] nitrogenous organics
		negative charged low MW fraction	[illegible] carbohydrates, [illegible] nitrogenous organics contained most of color and carboxyl groups
	riverwater organics	neutral charged fraction	[illegible] carbohydrates
Povoledo and Gerletti ([illegible])	lake sediment fulvic acid	unfractionated	[illegible] carbohydrates [illegible] hydrolyzable amino acids
		high MW excluded from G-25 low MW included in G-25	contains half of carbohydrates and proteins contains most of the color, fluorescence and aromatic hydroxyls
Rashid and King ([illegible])	marine sediment humic acid	high MW excluded from G-200	[illegible] meq/g ([illegible] meq/gC) carboxyl groups [illegible] meq/g aromatic hydroxyl groups [illegible] nitrogenous organics
		low MW included in G-25	[illegible] meq/g ([illegible] meq/gC) carboxyl groups [illegible] meq/g aromatic hydroxyl groups [illegible] nitrogenous organics
Reuter and Perdue ([illegible])	river fulvic acid	high MW excluded from G-10 low MW included in G-10	[illegible] meq/g carboxyl groups [illegible] meq/g carboxyl groups

developed in the soil science (Schnitzer and Kahn, 1972). Many tests, however, give arbitrary results and the outcome of the carboxyl and aromatic hydroxyl test, for example, is dependent upon the generally unknown spectrum of dissociation constants (Van Dyk, 1966). The colorimetric tests selected in the present study for functional group analyses may therefore be less arbitrary. All of the studies using samples from widely different environments, indicate a similar distribution of specific organics in the different molecular weight fractions. This may indicate that similar bacterial or chemical processes are governing the composition of the organics in natural environments. Several studies do indicate that bacterial processes are the most important. Identification of individual sugars in soil polysaccharides, for example, showed that xylose and glucose were present in lower concentrations than in the original plant material, while galactose, mannose, arabinose and uronic acid were present in higher concentrations. This relative distribution indicates that most of the sugars are derived from microorganisms (Forsyth, 1950). The presence of relatively large amounts of amino acids in the high molecular weight fraction is probably the result of excreted bacterial amino acids that preferentially attach to this fraction. Broadbent (1960) noted that labelled inorganic nitrogen and organic carbon often appear in the high molecular weight humic fraction as acid hydrolyzable amino acids. The aromatic compounds present in the 500 MW UF retentate may have been excreted by fungi and bacteria as orsellinic acid, salicylic acid, hydroxybenzoic acid and phenols and condensed to larger molecules as a result of the pH increase of the solution (Hoak, 1961).

MOLECULAR WEIGHT DISTRIBUTION

Related studies that used dialysis or ultrafiltration membranes as listed in table 7 and 8 were plotted as percentage of the organic matter retained versus nominal molecular weight cutoff (Figure 14). The results in Figure 14 indicate that the majority of the aquatic organics have molecular weights ranging from 10,000 to 100,000.

No systematic differences were noted between the molecular weight distributions determined by dialysis or membrane ultrafiltration. As compared with the other studies, the samples analyzed in the present study have the largest percentage of low molecular weight organics. This difference is mainly due to the large fraction of free volatile fatty acids generated during anaerobic acid fermentation. Since most of the investigations in figure 14 analyzed aerobic stabilized organics, the absence of low molecular weight organics is to be expected.

STABILITY OF ORGANICS IN DIFFERENT MOLECULAR WEIGHT FRACTIONS

Bacterially derived organics are subject to degradation. Degens et al. (1964), for example, noted a rapid decrease of carbohydrates with increasing time of burial or increasing depth in sediments while a less rapid decrease was observed for amino acids. No reduction in amount of humic substances was noted, while aromatic compounds sometimes showed an increase with depth of burial. Ishiwatari (1971) noted that carbohydrates showed the largest relative decrease with increasing depth of burial while hydrolyzable amino acids experienced a less rapid reduction. Humic acids did not show any decrease and in fact showed some increase. Swain (1970) also noted a gradual decrease of the carbohydrate content with increasing depths in lake sediments and an occasional increase of the organic nitrogen followed by a subsequent decrease. Aromatic compounds are generally less subject to decomposition than aliphatic compounds such as peptides and carbohydrates, and incorporation of proteins and carbohydrates in aromatic hydroxyl compounds renders the complex less susceptible to bacterial degradation (Benoit and Starkey, 1968). The results of these studies therefore agree with the present findings in that increased stability was noted for: free volatile fatty acids < carbohydrates < proteins < aromatic hydroxyls.

The more rapid decrease of the high molecular weight humic-carbohydrate-like material as compared to fulvic acid-like material as found in the

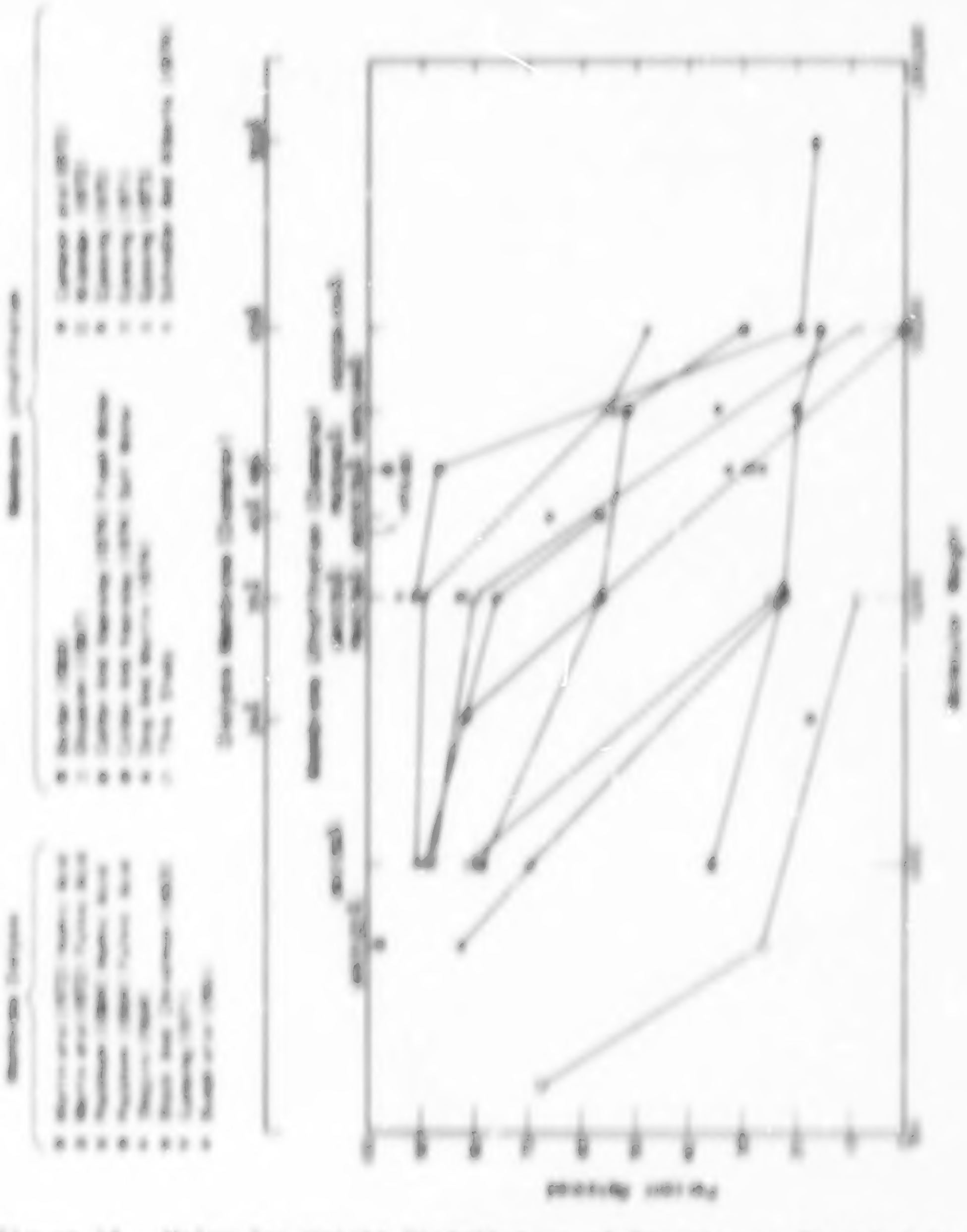

Figure 14. Molecular Weight Distribution of Organics as Reported in Different Studies and Determined by Dialysis and Membrane Ultrafiltration.

present study may be due to aerobic biological stabilization as the ORP in the leachate increased from -200 mv to about +200 mv with increasing age of the landfill. As the high molecular weight fraction only decreased in magnitude but not in relative composition (percentage carbohydrates and amino acids), one may conclude that entire parts of the high molecular weight molecule were hydrolysed which then become more susceptible to further enzymatic and bacterial degradation. The more rapid decrease of the high molecular weight fraction as compared to the fulvic like materials is probably not due to its preferential coagulation, since no correlation could be established between the magnitude of this fraction and the concentration of Ca and Fe which could have caused such coagulations. Preferential leaching to the subsoil, is also not likely to cause a more rapid decrease in the high molecular weight fraction since such leaching would rather cause a decrease of the low molecular weight fulvic-like material.

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IV

THE SEQUENTIAL REMOVAL OF ORGANIC MATTER IN LEACHATE DURING AEROBIC BIOLOGICAL DEGRADATION

CONCLUSIONS

The present study showed the sequential uptake of different classes of organics during aerobic biological degradation of leachate obtained from a recently installed fill. Using RO and UF membrane fractionation followed by specific organic analysis it was found that the leachate degradation occurs in four phases. The first phase was characterized by a removal of high molecular weight humic carbohydrate-like organics as measured by gelpermeation chromatography and specific organic analysis. Its removal may be due to adsorption onto bacterial flocs and by bridging single particles thus causing a decrease in the filtered turbidity. The second phase is characterized by the decrease of the free volatile fatty acids. This is also reflected in a decrease of the oxidation reduction potential, conductivity and dissolved oxygen. During the second phase of the fatty acid utilization, an accumulation of intermediates containing the carbonyl group and amino acids was detected and are most likely derived from the intracellular amino acid pool of the bacteria. After removal of these intermediates, representing the third phase of substrate removal,high-molecular-weight carbohydrates are excreted by the bacteria resulting in the further flocculation of the bacteria. The fourth phase consists of the removal of these high-molecular-weight humic-carbohydrate-like organics. The rate of organic matter removal and the amount removed gradually decreases when the removal progresses to phase four. The control mechanism that governs the sequential utilization appears to be catabolite inhibition, i.e., inhibition by the catabolite on the functioning rather than on the synthesis of the inducible enzymes. The present study also noted increasing stability for fatty acids, amino acids, carbohydrates, humic and fulvic acids. Since a similar sequence is noted in the relative composition of leachate samples from landfills of increasing age, only leachate from recently installed fills, containing relatively large amounts of fatty acids, will be amenable to biological treatment, while such methods will be less effective when the leachate contains large amounts of humic and fulvic acids.

INTRODUCTION

Organic matter analysis of leachate samples collected from solid waste landfills of different ages showed that the free volatile fatty acid fraction gradually decreased while organics such a humic-carbohydrate-like compounds and fulvic-like materials became more predominant with increasing age of the fill (Chian and DeWalle, 1976). The organic matter composition in leachate is expected to have a large impact on the treatment efficiency of biological units treating such waste since some classes of organics are more biodegradable than others. Several pilot- and full-scale aerobic and anaerobic units treating leachate have been in operation but consistently high efficiency was generally not obtained due to changes in strength and variations in composition of the leachate (Ham *et al.*, 1976). It was therefore the purpose of this study to follow the removal of specific classes of organics in leachate during aerobic biological degradation. Such tests simulate the organic matter removal in a plug-flow activated sludge unit treating leachate.

It is well known that microorganisms in a completely-mixed reactor are more efficient in the removal of single substrates than a plug-flow reactor (Herbert *et al.*, 1956). The completely-mixed reactor is preferred because of the autocatalytic nature of the reaction and the apparent zero order kinetics for single substrate utilization. However, with a complex multi-substrate medium such as wastewater, the plug-flow reactor, particularly with sludge recycle, is preferred (Eckenfelder and O'Connor, 1961). Since uptake rates of individual substrates may be markedly different in the presence of other substrates, the overall reaction kinetics may no longer be zero order (Mateles and Chian, 1969). Well-known examples of such substrate interaction is the occurrence of diauxic growth in batch pure culture (Monod, 1949), sequential substrate utilization in batch mixed cultures (Gaudy, 1962; Gaudy *et al.*, 1963a; Gaudy *et al.*, 1963b; Gaudy *et al.* 1964; Stumm-Zollinger, 1968) and sequential substrate removal in continuous pure or mixed cultures

(Komolrit and Gaudy, 1966; Ghosh and Pohland, 1972). All of the above studies, however, were conducted using mixtures of pure carbon substrates. In no instance was the substrate comprised of more than two or three different carbon sources. There have been no reported studies on the sequential uptake of substrates by microorganisms in naturally occurring wastewaters known to contain multiple substrates (Painter, 1971). The study reported herein demonstrates the sequential uptake by microorganisms of the different chemical groups present in leachate using batch-mixed cultures. The leachate was generated from a pilot-scale sanitary landfill. Results of this study show that the same control mechanisms that govern the sequential uptake of substrates by bacterial cells in well-defined synthetic media are also applicable to complex wastewaters.

The efficiency of activated sludge systems depends on the ability of microorganisms to degrade the different food sources present in the influent. The carbon leaving the effluent of an activated sludge unit is mostly refractory or inert material that the bacteria are not able to degrade. It may consist of polymerized waste products of the bacteria which are excreted into the solution. Other refractory materials may consist of cell material or inert material from lyzed cells. Some of these slowly degradable products have been referred to as humus-like substances and their presence may represent 20 to 50 percent of the chemical oxygen demand (COD) of the effluent. These humic substances have a molecular weight ranging from several hundreds to tens of thousands (Schnitzer and Kahn, 1972). They are able to chelate heavy metals and prevent their removal in treatment plants, protect pathogenic organisms by coating and impair the color of the effluent.

Only a few studies have analyzed the organic composition of the effluent of biological treatment plants. Painter et al. (1961) were able to classify 25 percent of the organic matter while Bunch et al. (1961) identified 35 percent. The remaining part was thought to consist of humic material. Dialysis tests with secondary effluent showed that 21 to 49 percent of the soluble COD was retained by a cellulose dialysis membrane indicating presence of high molecular compounds (Bunch et al., 1961).

Rebhun and Manka (1971) calculated that 39.3 to 45.2 percent of the COD in activated sludge effluent consisted of humic substances; however, their extraction technique may have overestimated the fulvic acid fraction. Free volatile fatty acid and other compounds could be extracted by the butanol used for extracting the fulvic fraction which, in turn, may increase the weight of this layer.

A preliminary study by Bender et al. (1970), using gel-permeation chromatography, showed the presence of two molecular weight fractions in activated sludge effluent. Both fractions had a complexing capacity for heavy metals. A similar technique employing Sephadex columns was used by Obiaga and Ganczarczyk (19) and Minear and Christman (1966) who also found a low molecular weight fraction in the effluent.

A recent development in the identification of organic matter in wastewater is the combined use of membranes and Sephadex columns to analyze the higher molecular weight substances. Goldsmith et al. (1971) first characterized the molecular weight fractions of proteins in cheese whey using Sephadex columns while employing membrane ultrafiltration to concentrate the high-molecular-weight protein fraction. A similar technique was used by A. D. Little, Inc. (1971). No use has yet been made of reverse osmosis and ultrafiltration membranes to concentrate wastewater or effluent for analyzing organic matter. In previous studies in this laboratory, initial use was made of extraction techniques to obtain the high molecular weight humic and fulvic acids. Because of the unsatisfactory nature of these procedures, new methods were adopted and extensive use was made of ultrafiltration (UF) and reverse osmosis (RO) membranes in combination with Sephadex separation (DeWalle and Chian, 1974).

A relatively large number of studies concerning the nature of refractory organics have been conducted in the area of soil biochemistry and recent reports show humic acid to have a molecular weight ranging from 5,000 to 100,000 while the molecular weight for fulvic acid varied between 2,000 and 10,000 (Hurst and Burges, 1967). They also showed that about 50 percent of the total carbon of humic acid might be present in aromatic structures, 20 percent in functional groups and 30 percent in aliphatic structures. A large fraction of the oxygen is contained in the functional groups especially the carboxyl group which can be as high as 8.9 meq/g for fulvic acid and 3.7 meq/g for humic acid.

The formation of humic and fulvic acids is not yet well understood. Several investigators have proposed that fungi and bacteria are able to convert not yet stabilized organic material into final humic and fulvic matter. Waksman (1936) formulated that humic substances are derived primarily from the interaction of plant lignin with proteins from the soil microorganisms which generates a humic nucleus to which undecomposed residues are attached. An opposing view has been presented by Kononova (1966) who indicated that some soil fungi would convert a food source to simple phenols, amino acids and carbohydrates which then condensed extracellularly into a brown organic residue which was found to closely resemble natural humic acid. The author presented evidence for quinoid-amino acid melanoidin type humic compounds.

Similar studies were conducted by Martin and Haider (1969) and Hurst and Wagner (1972) who grew fungi on a glucose-asparagine-salt solution for several weeks and detected numerous phenolic compounds in the medium. After the cessation of the excretion of phenolic compounds, a humic-like polymer appeared in the medium accompanied by a decrease in the free phenolic compounds. The humic acid was similar to soil humic acids in its slow rate of microbial decomposition, number of carboxyl and phenolic hydroxyl groups and molecular weight as determined by gel filtration. The humic substances may have been formed intracellularly and released into the solution after cell lysis.

Besides the relatively stable humic and fulvic acids, it has been found that certain high-molecular-weight carbohydrates, alone or in combination with humic material, are also resistant to microbial attack. The most stable compounds were isolated from exocellular polysaccharides (Finch *et al*., 1972; Barker and Somers, 1970). These compounds, excreted at the end of the logarithmic growth phase, may have an important function in polymer bridging of bacterial cells resulting in floc formation (McKinney, 1953; Dunican and Seeley, 1965; Friedman *et al*., 1969). Bush and Stumm (1968)definitely demonstrated the flocculation properties of this exocellular polysaccharide. Pavoni *et al*. (1972) came to a similar conclusion. In addition, they found large amounts of nucleic acid present in this polymer. Both studies did not indicate, however, how much of this polymeric material is released in the effluent of an activated sludge unit. Peter and Wuhrman (1971) stated that humic acid, isolated in substantial quantities in the effluent, might have a function in bridging cell particles and subsequent floc formation.

All of the above studies reported little data concerning the amount of humic substances in activated sludge effluent. None of the studies reported at what stage during the growth phase of the microorganism these substances were formed, and which factors would govern the release of these substances in the solution. To test the rate of formation of humic substances, a complex leachate was added as food source to a bacterial batch culture. The refractory materials were measured at different stages during the degradation of the food source using membrane and Sephadex concentration and separation methods. Leachate from pilot-scale lysimeter was used since it represented a waste stream containing complex organic constituents and a low energy source, which would have a more pr[illegible] effect on the formation of the humic material.

MATERIALS AND METHODS

The batch aeration tests were conducted in an 80 ℓ vessel filled with 60 ℓ of diluted leachate. Leachate, representative of a young landfill, was obtained from a pilot-scale lysimeter filled with ground solid waste and was extensively analyzed for its organic and inorganic constituents. The bacteria culture was obtained from an activated sludge unit receiving both municipal sewage and leachate at a concentration of a half volume percent of the waste stream. Air was supplied to the mixture at a rate of 2.8 ℓ/min. The first aeration test (A) was conducted with a leachate diluted to a concentration of 91 mg/l TOC and an added bacterial density of 20 mg/l MLSS. In the second run (B) both constituents were increased approximately five fold and in the third test (C) the leachate concentration was double the amount used in the second test. The leachate and the bacteria were diluted with distilled deionized water supplied with N and P in similar quantities as used in BOD dilution water (APHA, 1971).

At the end of each test run the solids were removed by centrifugation and the centrifugate was concentrated with an Abcor (Cambridge, Mass.) BEU-301 reverse osmosis unit using an AS-197 membrane.

The higher molecular weight organic matter was further concentrated with an Amicon (Lexington, Mass.) ultrafilter cell using an UM-05 untrafiltration membrane with a nominal 500 MW cut-off. The retentate was separated into its components using Sephadex G-75 (Pharmacia, Piscataway, N.J.) columns which separate carbohydrate-like material between a molecular weight of 1,000 and 50,000. The high-molecular-weight fraction of the G-75 was further separated with a G-150 column and the low molecular weight fraction with a G-25 column (Gjessing and Lee, 1967). The total organic carbon (TOC) analysis established the distribution of the organic matter in the different molecular weight fractions. Proteins in the different molecular weight fractions were measured with the ninhydrin method after extensive digestion (Stevenson and Cheng, 1970). The more sentivive Lowry test could not be used, since many constituents with a phenolic hydroxyl group, such as humic and fulvic acids, interfere (1955). The carbohydrates were determined with the anthrone test (Dreywood, 1946).

Functional groups in the different molecular weight fractions were also measured with colorimetric tests. The carbonyl groups were determined with 2,4-dinitrophenylhydrazine, which detects ketones but does not measure carbonyl in carboxyl groups or aldehydes in sugars, as described by Lappin and Clark (1951) and modified by Sanders and Schubert (1971). The carboxyl groups were determined with the hydroxyl amine test as reported by Montgomery *et al.* (1962) and Lehmann and Wilhelm (1968). The phenolic hydroxyl group was determined with the tannin test as reported in Standard Methods (APHA, 1971). These colorimetric tests were also conducted on filtered samples using 0.45 μ Millipore (Bedford, Mass.) membrane filter. Samples were removed at regular intervals during the aeration test to determine changes in the monitored parameters over time. Other parameters monitored during the aeration test were turbidity, after filtration through a Whatmann 4 filter paper (W and R Ldt, Madestone, G. B.), suspended solids, pH, dissolved oxygen (DO), oxidation-reduction potential (ROP), conductivity, and total organic carbon (TOC).

RESULTS AND DISCUSSION

An extensive analysis of the leachate used in this study showed that 61 percent of the TOC consisted of free volatile fatty acids with butyric and acetic acid present in the highest concentrations followed in concentration by caproic acid, iso-valeric acid, propionic acid, valeric acid and isobutyric acid, respectively.

The high-molecular-weight fraction, as obtained in the ultrafilter retentate, represented 27.2 percent of the TOC of the leachate. When the retentate was further separated on a Sephadex G-75 column, two large peaks, as measured with the TOC, became apparent (Figure 15). The last eluted peak representing the low-molecular-weight material (69 percent of the TOC of the UF retentate) evolved before the salt peak. It was, therefore, considered to have a molecular weight larger than 1000, possibly between 1000 and 5000. The high-molecular-weight material representing 21.6 percent of the TOC of the ultrafilter retentate evolved at an elution volume similar to that of the Sephadex 10^6 MW grade dextran, and is, therefore, larger than 50,000. The application to G-25 and G-150 did not result in further significant separation. A similar result was observed by Gjessing and Lee (1967). Analysis showed that the carbohydrates, phenolic, hydroxyl and carbonyl groups were equally distributed between the two fractions; however, the carboxyls were only observed in the low molecular fraction. Infra-red spectra of the two molecular-weight fractions showed close resemblance to humic and fulvic acid spectra as reported by Goh and Stevenson (1971) and Stevenson and Goh (1971). The high molecular weight fraction resembled a humic acid with a strong peak near 1620 cm^{-1}, which corresponds to the carbonyl adsorption bond in quinones and diketones. Also, it had some characteristics of a polysaccharide. The low molecular weight fraction resembled a fulvic acid with a strong peak near 1720 cm^{-1}, which corruborated the result of the carboxyl test. Although

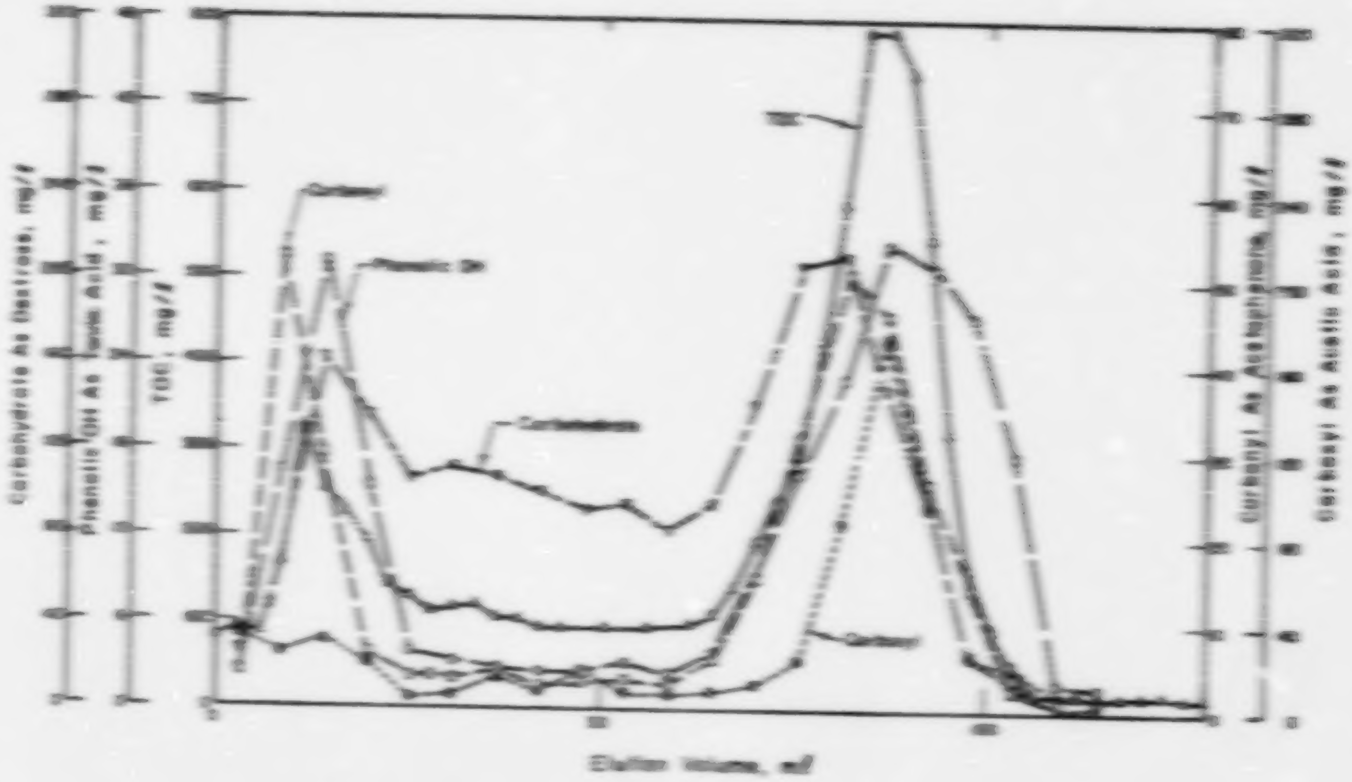

Figure 35. Elution Pattern of the Ultrafiltration Retentate on Sephadex G-75 before Aeration of the Leachate with Activated Sludge

further characterization studies with mass spectrometry are still underway, the high-molecular-weight substance will be referred to as humic acid (HA) and the low-molecular-weight fraction as fulvic acid (FA).

The unidentified fraction of the organic matter (21.8 percent of the TOC) may have consisted of fulvic acid fragments with a molecular weight less than 500 as detected in the UF permeate. This fraction gave a positive phenolic hydroxyl test. Most of the inorganic anions were present as chlorides and sulfates. Calcium, iron and sodium were the major cations.

The result of the aeration tests showed that organics in the leachate, as measured with the TOC, were removed in four steps; the first mainly representing the removal of the humic, carbohydrate-like substances; the second representing the uptake of the free volatile fatty acids; and the third corresponding to the removal of intermediates released during the second stage, while refractory organics are removed in the fourth stage.

In order to gain insight into the mechanism of the formation of refractory material, test A was terminated at the beginning of the second phase during maximum fatty acid uptake. Test B was terminated at the end of the third phase, when the excreted intermediates were exhausted (53 hrs, see Figure 18). The third test (C) was aerated for 450 hours.

The changes of the different parameters during aeration of test B are presented in Figure 16, 17 and 18. Figure 16 shows that the organic matter was removed in sequence,with the first phase occurring between zero and 10 hours; the second phase between 10 and 23 hours; and the last phase between 23 and 48 hours. In the first phase, it shows a decrease in TOC from 580 mg/l to 480 mg/l. In this period the suspended solids show their first increase (Figure 17). An increase was also observed in the ORP, pH and conductivity, but no large oxygen demand was exerted. The carboxyl test measuring the free volatile fatty acids

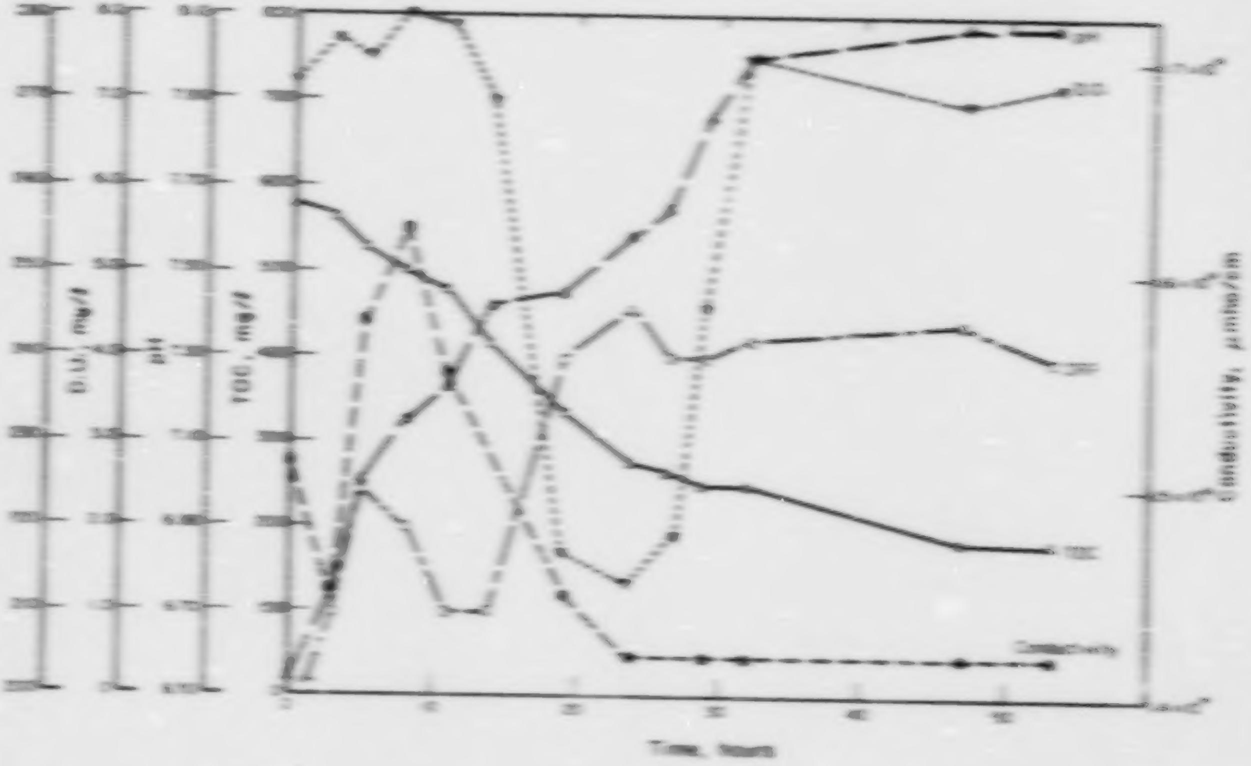

Figure 16. Change of pH, DO, ORP, TOC and Conductivity During Aeration of the Wastewater

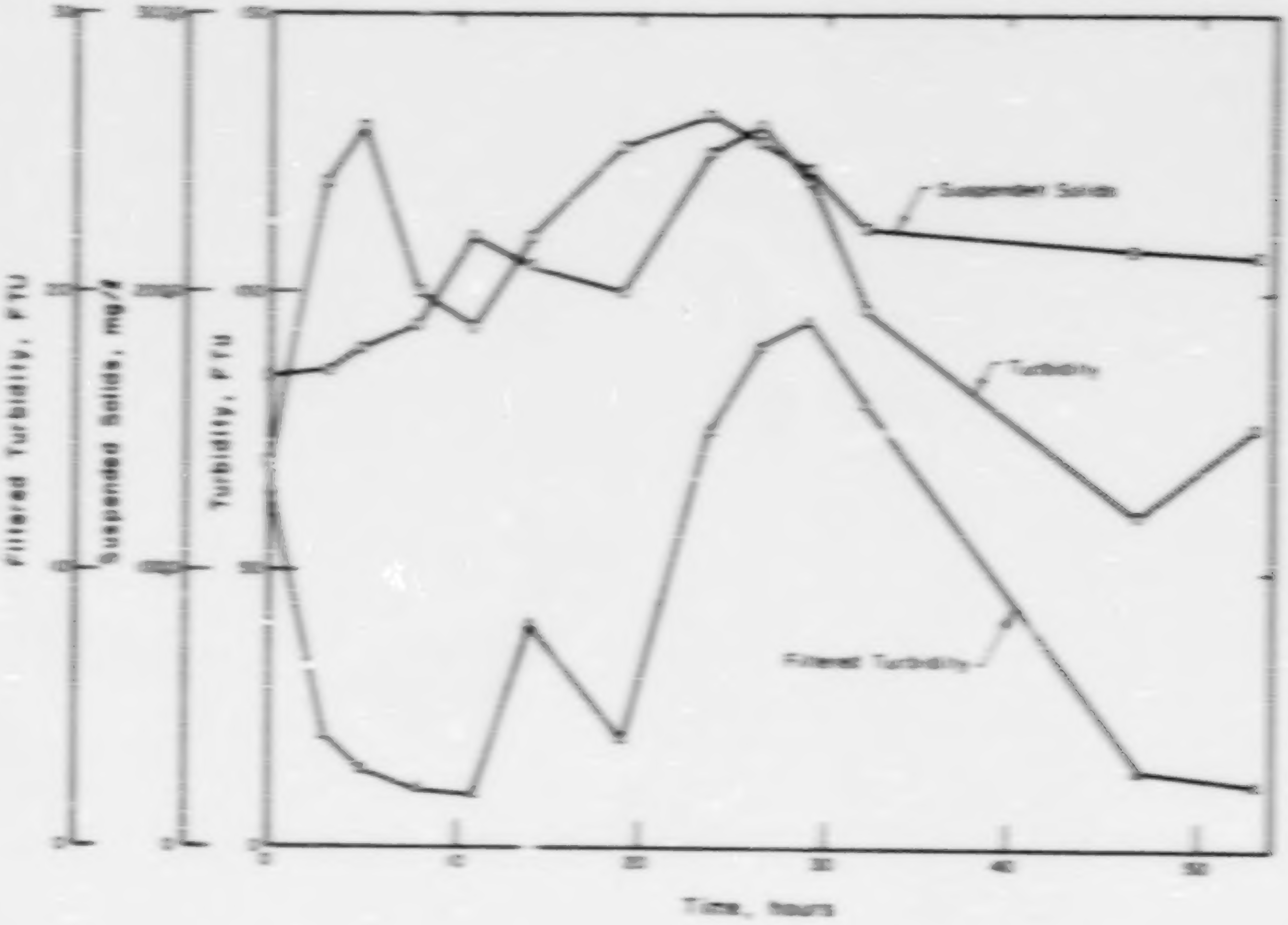

Figure 17. Change of Suspended Solids, Turbidity and Filtered Turbidity During Aeration of the Wastewater

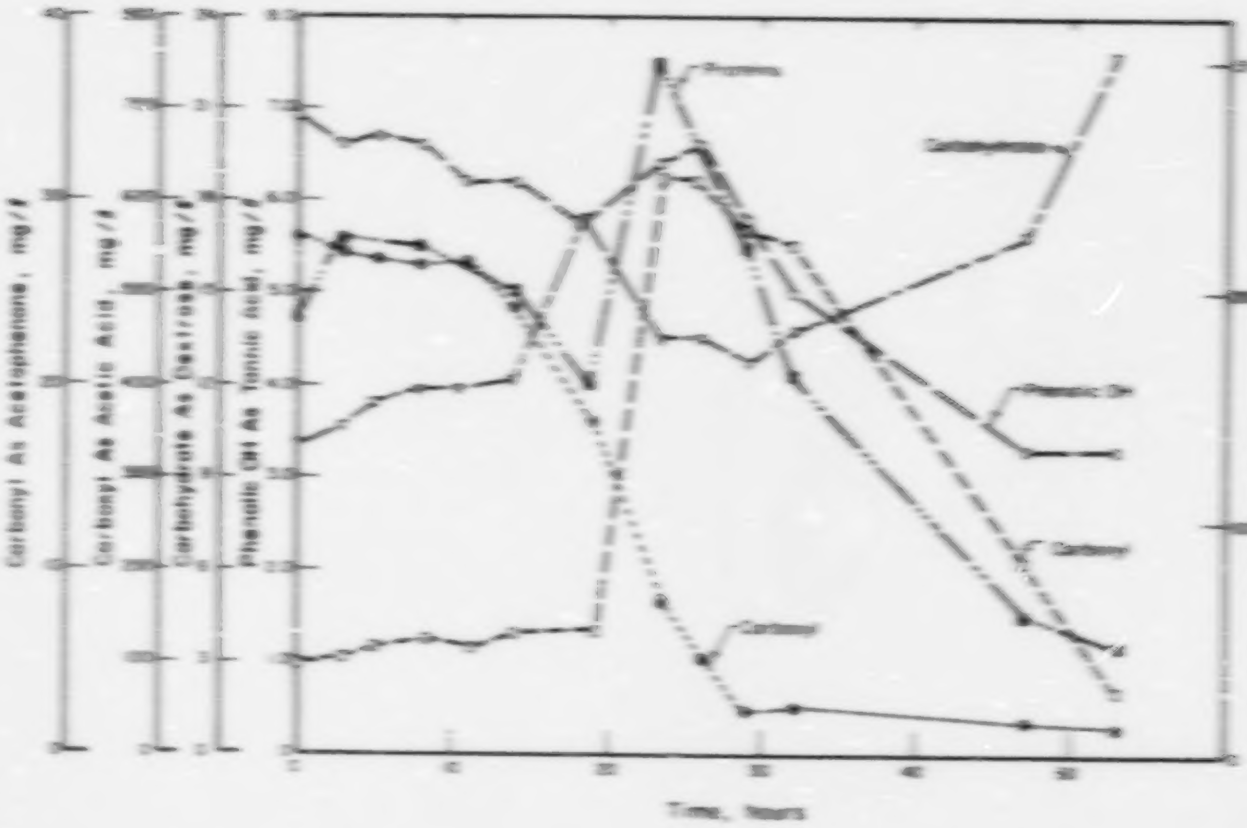

Figure 18. Change of Proteins, Carbohydrates, Phenolic Hydroxyl-, Carbonyl-and Carboxyl Substances During Aeration of the Wastewater

present in the diluted leachate also remained constant (Figure 18), while the increase in conductivity indicated dissociation of these acids as the pH increases.

The analysis of the humic material, obtained after termination of test A with subsequent RO and UF concentration of the effluent, showed that the amount of substances greater than 500 MW had dropped from 27.2 percent of the initial TOC to 7.10 percent of the TOC of the solution at end of test A. Of the different components detected in the 500 MW UF retentate, a large reduction was observed in the carbohydrates. The ratio of the carbohydrates to TOC (as mg dextrose/mg TOC) in the humic acid and fulvic acid peak decreased from 0.47 to 0.35 and from 0.23 to 0.16 respectively (Figure 16 and 19). These ratios were obtained using the carbohydrate value divided by the maximum TOC value of the fraction. A similar decrease in ratios was observed for the phenolic hydroxyl and carbonyl in the humic acid fraction. The increase in carboxyl/TOC ratio may indicate the higher resistance of this group to bacterial attack or a conversion from hydroxyl and carbonyl groups to the carboxyl group. During this first stage the fulvic acid is removed to a larger extent than the humic acid fraction with the result that the ratio of humic to fulvic increased from 0.42 to 0.77. This preferential removal may be due to the higher initial concentrations of the fulvic compared to the humic acid. The rapid increase in suspended solids and a decrease of the filtered turbidity during the first 5 hour period (Figure 17) may indicate that the humic-carbohydrate-like materials have flocculating properties and are able to bridge single particles. These data may therefore indicate that the humic carbohydrate-like materials are removed by adsorption onto the bacteria flocs. The presence of a high iron concentration (75 mg/l) and its oxidation during aeration may also have enhanced the floc formation.

It should be noted that the filtered turbidity (Figure 17) and the carbohydrates (Figure 16) show a small increase at the end of the first, and the beginning of the second phase. It could well be that at this

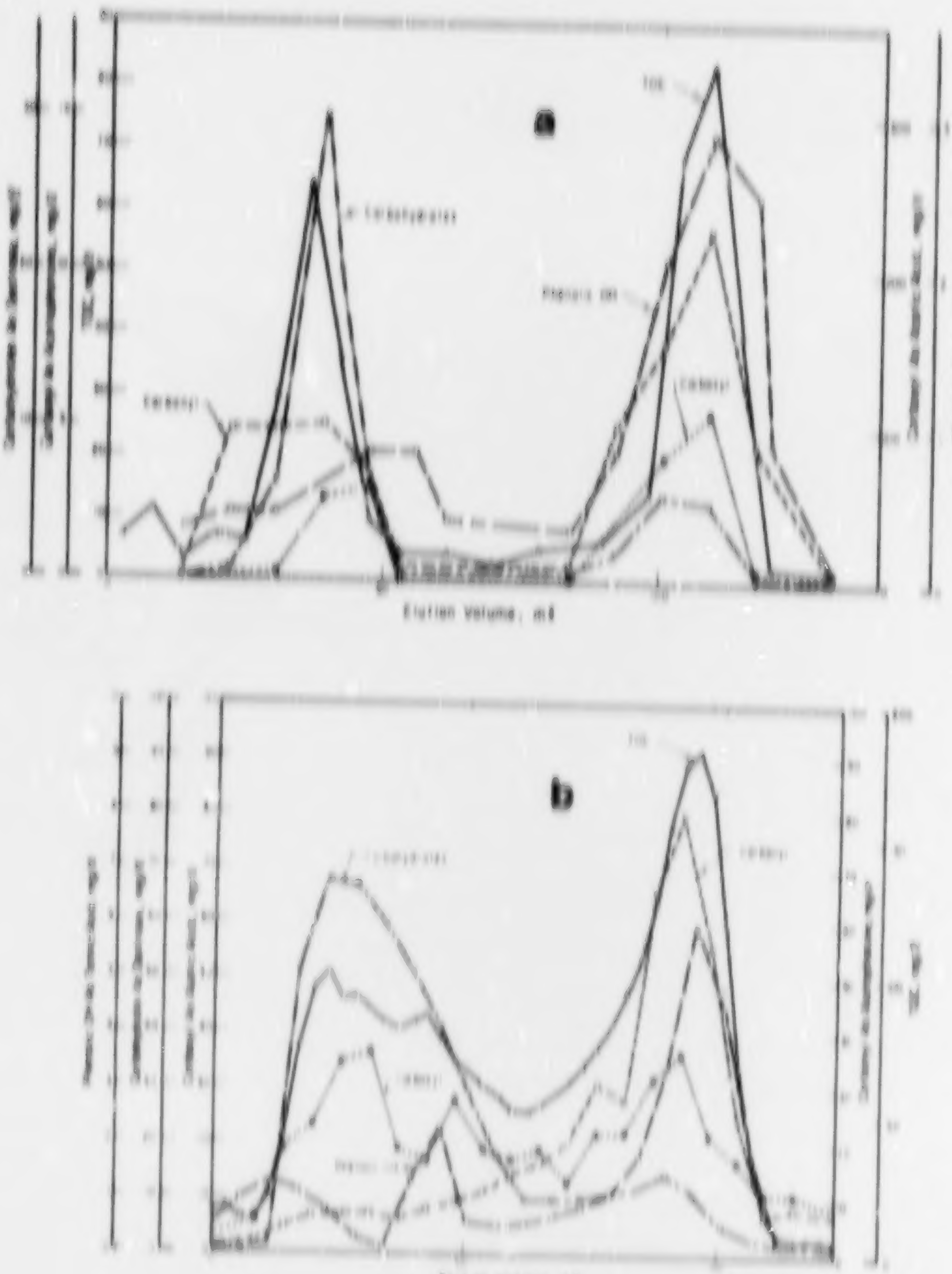

Figure 19. Elution Pattern of the Reverse Osmosis and Ultrafiltration Retentate on Suphadex G-75 During Aeration of the Leachate
(a) Corresponding with Maximum Substrate Uptake
(b) After Uptake of the Readily Available Carbon Source

point the degradable part of the adsorbed humic carbohydrate-like materials is consumed by the cells and that some of these degraded carbohydrate segments are subsequently released into the solution (Figure 18). Redispersion of the flocculated particles due to decrease of such bridging materials is evidenced by the increase in the filtered turbidity (Figure 17). Utilization of carbohydrates in the first phase is also evidenced by the inhibition of the fatty acid uptake (Figure 17). This was shown by Chian and Mateles (1968) to occur with butyric acid in the presence of glucose. The uptake rate of monosaccharides by the cells occurs more rapidly than that of fatty acids. The lower rate of substrate utilization observed in the first phase as compared to the second phase fatty acid utilization is, therefore, most satisfactorily explained by the rate limiting step of hydrolysis. It is necessary to hydrolyze the high-molecular-weight carbohydrates in the media to make them available to the cells (Banerji *et al.*, 1968).

The second phase of the TOC removal is characterized by the decrease of the free volatile fatty acids as measured with the carboxyl test (Figure 18). Shortly before the beginning of the acid uptake the oxidation reduction potential (Figure 16) shows a decrease, indicating the decrease of the oxidation state of the oxidative co-enzymes. The decrease in conductivity parallels the actual removal of the fatty acids from the solution. Only after the acid is taken up in the Tricarboxylic Acid Cycle (TCA cycle) is an oxygen demand exerted which explains the decrease of the DO in the solution lagging behind the actual uptake.

During the second phase of the fatty acid utilization, an accumulation of an intermediate containing the carbonyl group was first detected in the medium after about 19 hours of aeration (Figure 17). A similar accumulation was observed for proteins or amino acids at a much higher concentration. The excretion of intermediates during the active metabolism of monosaccharides was realized by Gaudy *et al.*(1964) Mateles and Chian,(1969). The intermediates were later found to contain acetic acid. These authors observed that as much as 30-40% of the carbon substrates, particularly when fed in the form of mixtures, was excreted as intermediates. However, only 10% of the carbon substrates lost as intermediates was identified as acetic acid when fed with glucose alone, and 15% when fed with glucose and lactose. A complete material balance of the excreted intermediaries has never been attained due to the lack of information on the nature of other excreted compounds (Mateles and Chian, 1969).

While intracellular amino acid pool accumulation has been reported in batch cultures of Saccharomyces cerevisiae (Brown, 1969; Moat et al., 1969) Bacillus megaterium (Nelson and Kornberg, 1970), and Escherichia coli (Brada, 1970), only one study reported the accumulation of extracellular amino acids. Clark et al. (1972) observed such an increase in cultures of Bacillus licheniformis when the cells changed from vegetative to sporulation metabolism. Most of the authors observed that the total intracellular amino acid pool increased during exponential growth and decreased rapidly thereafter. This agrees well with this observation which shows that the extracellular proteins (or amino acids) decrease rapidly toward the end of the active metabolism of fatty acids. It was calculated that the maximum amount of proteins excreted accounted for 35-50% of the total weight of the fatty acids initially present in the medium, which is appreciable (Figures 18).

The authors cited above also reported that only a few amino acids made up the majority of the pool. Between 70-80% of the amino acids in the pool consisted of glutamate followed by 10-20% alanine and in some cases small amounts of aspartate. A review of amino acid biosynthesis shows that each of these amino acids is derived from an α-keto acid (α-ketoglutaric acid, pyruvic acid and oxaloacetic acid, respectively). Only glutamic acid is formed from direct amidation of α-ketoglurarić acid with ammonia. Aspartic acid and alanine are formed by transamination of oxaloacetic acid and pyruvate with glutamic acid. The abundance of glutamic acid found in the amino acid pool as reported by the above authors is therefore well explicable.

Based on these observations, it was expected that the excreted amino acids and keto containing compounds found in this study were predominatly glutamate and glutamine, respectively. Excretion of substantial amounts of alanine during active metabolism of fatty acids is unlikely, since pyruvate is not an intermediate in the catabolic pathways of fatty acids. Excretion of the other α-keto acids, such as α-keto glutaric acid and oxaloacetic acid, is also unlikely since the intermediaries in the catabolic pathway are not expected to accumulate in substantial amounts in the cells. It is worthwhile to note that both acetic acid and glutamate represent intermediates being excreted at the branching point of the metabolic pathways. The former is excreted prior to acetyl-CoA entering into the citric acid (Krebs) cycle for energy yielding steps of oxidative phosphorylation for ATP production. Both of these excretions appear to be regulated by the delicate enzyme control systems within cells to prevent from unnecessary waste of energy.

As shown in Figure 16, the rate of TOC removal started decreasing at about the twenty-ninth hour of aeration. At this time, the dissolved oxygen (DO) level increased rapidly as a result of exhaustion of fatty acids. This is indicated by the diminishing of the carboxyl group in the medium (Figure 18). This transition into a slower rate of substrate uptake in terms of TOC removal represents the third phase of substrate utilization. In this phase, continuing removal of excreted proteins (amino acids), carbonyl (keto)-containing and phenolic-OH containing substances were observed (Figure 18).

During the removal of these intermediates in the third phase, the carbohydrate concentration starts to increase again. This also corresponds with a decrease in the filtered turbidity. The increase in the carbohydrate concentration reflects an increase in the amount of refractory material. After termination of test B, the solids were removed by centrifugation. The supernatant was concentrated with the RO and UF module. Results of TOC analysis showed that the amount of organic matter retained by the 500 MW ultrafiltration membrane had increased from 7.1 percent to 21.6 percent. This represents an actual increase in concentration of the refractory material in the solution from an estimated 20.7 mg/l during the middle of the second phase to 39.9 mg/l at the end of the third phase. The most notable increase was shown by the carbohydrate in the larger than 50,000 MW fraction (Figure 19). As a result of this increase, the carbohydrate to TOC ratio of the humic acid fraction increased from 0.35 to 0.67.

The fulvic acid fraction was characterized by a strong increase in carbonyl-bearing structures as shown at an elution volume of 90 ml (Figure 19). This appears to be at an expense of a reduction of the carboxyl groups, since the ratio of carboxyl to TOC (as mg acetic acid/mg TOC) decreased from 1.32 at the end of test A to 0.158 at the termination of test B. It is also seen from Figure 19 a and b that the humic to fulvic acid ratio decreased from 0.77 (Figure 19a) to 0.55 (Figure 19b). This may have been the result of the preferential adsorption and bridging of the humic carbohydrate-like polymers onto the bacteria during the flocculation stage. The fulvic fraction showed a decrease in the carbohydrate/TOC ratio from 0.16 in the middle of the second phase (test A) to 0.06 at the end of the third phase (test B). This lower carbohydrate content may have prevented its adsorption onto the sludge particles by not providing sufficient polymers for bridging to the bacterial mass.

This observation may also explain the settling behavior observed in a continuous control and test unit employed during further investigations. The test unit receiving various volume percentages of leachate and municipal sewage generally experienced poorer settling characteristics than the control unit treating only municipal sewage. The carbohydrate to TOC ratio of the humic acid fraction in the effluent of the control unit was 0.68 while it was only 0.37 in the effluent of test unit. This apparent shortage of humic-carbohydrate-like polymers and the resulting lack of bridging may have caused the observed impairment of the flocculation and the higher TOC and color in the effluent of the latter unit. A large part of this TOC, which mostly consists of humic substances, may not be able to adsorb onto the sludge cells and be removed along with the sludge. The higher humic acid to fulvic acid ratio in the effluent of the test unit as compared to the control unit would substantiate this conclusion. This observation has an important impact on activated sludge sewage treatment plants. These units are operated with respect to optimum carbon removal, which may differ from the conditions required for optimum floc formation and removal of humic substances. If more stringent requirements will apply to the presence of residual organics in the effluent, the treatment plant or its operation may have to be modified in order to ensure optimum removal of these substances.

A subsequent experiment (test C) was conducted with a feed containing approximately twice the amount of the leachate as used in the previous experiment and tested through a much longer period of aeration, 450 hours (Figure 20, 21). A decrease of the humic-carbohydrate-like materials produced by the cells along with the others can be seen clearly at a time starting from 160 hours of aeration (Figure 21). This represents the fourth phase of substrate utilization which is carried out at even a slower rate as is shown by the lower rate of TOC removal (Figure 20).

These four distinct phases of substrate utilization not only demonstrate clearly the occurrence of sequential substrate removal in a natural wastewater, but also explain well the shape of the inverse sigmoidal curve commonly observed for the BOD removal in the wastewater. Figure 20 depicts a typical inverse sigmoidal curve for the TOC removal from the wastewater under this study.

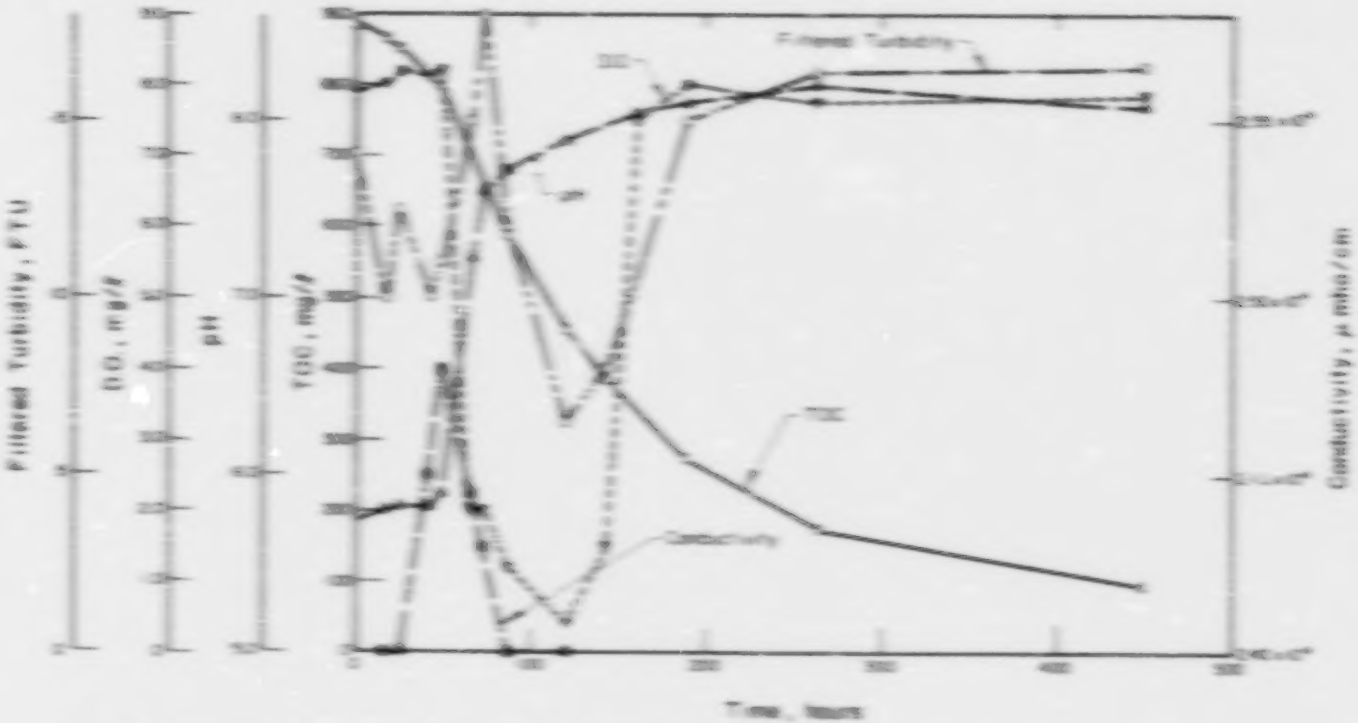

Figure 20. Change of Filtered Turbidity, DO, pH, TOC and Conductivity During a Longer Period of Aeration with a Higher Strength Leachate

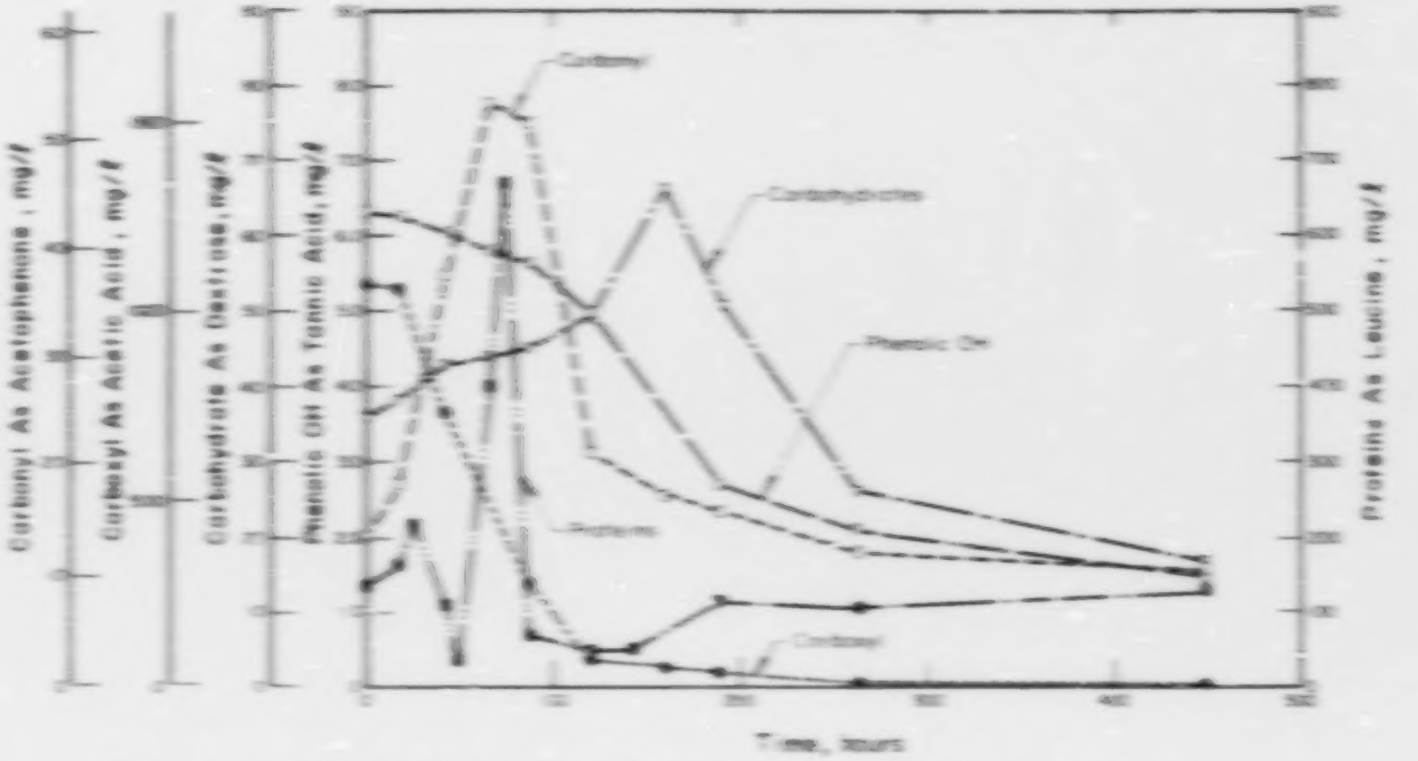

Figure 21. Change of Carbohydrate, Proteins (Amino acids) and Phenolic Hydroxyl-, Carbonyl- and Carboxyl Containing Substances During a Longer Period of Aeration with a Higher Strength Leachate

The overall control mechanism that governs the sequential utilization of substrates appears to follow the mechanisms described by Gaudy _et al._ (1964), i.e. catabolite inhibition, wherein the effect of the catabolites is on the functioning, rather than on the synthesis of the inducible enzymes. This is evidenced by the smooth and the continuing curve observed for TOC removal and the absence of the lag period required for the utilization of the less preferred substrate after the exhaustion of the more preferred one.

The present study shows that the relative composition of the substrate i.e., the leachate, has a large impact on the rate of organic matter removal and on the total amount removed. Increased stability is noted for fatty acids,amino acids, carbohydrates, humic and fulvic acids. As pointed out by Chian and DeWalle (1976) a similar sequence is noted in the relative composition of leachate samples collected from landfills of increasing age. This in turn indicates that leachate of young fills, containing substantial amounts of fatty acids, is amenable to biological degradation while such treatment methods will be less effective when the leachate contains large amounts of fulvic and humic acids. Such leachate is therefore preferably treated by physical-chemical methods.

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V

METALS AND THEIR INTERACTION WITH ORGANIC MATTER IN LEACHATE

CONCLUSIONS

It was noted in the present study that heavy metals are present in relatively high concentrations in landfill leachate. The determination of heavy metals by flame atomic absorption spectroscopy is subject to relatively large matrix interferences in heavily polluted leachate samples; this effect is less noticeable in more dilute samples. The matrix interference causes a reduction in the absorbance in the flame at the analyte wavelength resulting in a lower apparent metal concentration. It was observed that Cu was subjected to the largest matrix interferences followed by Cr, Ni, Pb, Cd and Zn respectively. The interfering effect can be eliminated by using the standard addition technique in which a sample is spiked with a known amount of the metal. The obtained recovery factor is than used to correct the measurement of the unspiked sample. It was noted that the interference in the Cr determination was both related to the absolute salt content and to the relative salt content (with respect to the heavy metal concentration), indicating that simple dilution of the polluted leachate sample will not totally eliminate the matrix interference. In addition it was noted that also Cl resulted in lower Cr recoveries as measured with the standard addition technique. The percentage depression in the Ni determination was best correlated with the absolute salt content and the concentration of Ca and Fe. The depression in the Cu determination may have been the result of the presence of phosphate compounds, while the Pb analyses showed that both a high relative salt content and the presence of sulfates decreased the recovery of the metal. It was further noted that La had to be added to the leachate samples as a releasing agent during the Ca determination.

Analysis of the heavy metal content in 12 leachate samples collected from landfills of different ages and subjected to varying stabilization and leaching periods, showed that the heavy metal content decreased more rapidly with age than other inorganic components. This is most likely the result of adsorption and precipitation reactions which in turn are enhanced by an oxidation reduction potential gradually increasing with increasing age of the landfill.

Extensive fractionation of the different molecular weight fractions present in leachate showed that the majority of the metals permeated the 500 MW UF membrane indicating that chelation of the metals by refractory organics is not a major mechanism in metal attenuation processes. A deviation of this general pattern was noted for iron which was associated for a considerable part with the larger than 100,000 MW UF retentate. Measurement of the ion exchange capacity and carboxyl group density indicated that only a small amount of the iron in this fraction can be chelated and that most of it is apparently present as ferric hydroxides. Membrane ultrafiltration of additional leachate samples showed that the percentage Ca and Fe retained with the 10,000 MW UF membrane increased with increasing magnitude of the high molecular weight organic fraction. The increase of the calcium content in the UF retentate may be due to chelation by the functional groups of the organic matter, while the increase in iron content is probably due to interaction of hydroxide complexes and the organics by adsorptive processes. The copper retention showed considerable competitive effects indicating that other metals such as Fe, Ca and Zn are better chelated. Treatability studies of leachate with aerobic and anaerobic biological systems showed that substantial amounts of the the metals are removed by these processes, again indicating that chelation is not a major factor. Removal in aerobic systems is likely due to formation of metal hydroxides and carbonates, while removal in anaerobic systems is the result of formation of insoluble metal sulfide and carbonate. Thus despite the relative high concentrations of heavy metals detected in leachate, no physical chemical pretreatment step is necessary to remove these metals prior to biological treatment.

INTRODUCTION

Concentration of Heavy Metals in Leachate

Leachates generated by infiltrating rainwater in landfills constructed from recently deposited solid waste will contain high concentrations of organic and inorganic pollutants. Merz (1954) measured maximum concentrations of 2570 mg/l calcium (Ca), 410 mg/l magnesium (Mg), and 305 mg/l iron (Fe) in leachate from a lysimeter filled with solid waste. The maximum sodium (Na) and potassium (K) concentrations of 1805 and 1860 mg/l, respectively, were approximately equal. The major anions were chloride (Cl) and sulfate (SO_4) with a maximum concentration of 2350 mg/l and 710 mg/l.

Qasim and Burchinal (1970) also found that Na, K, Ca, Mg, and Fe were the major cations. They showed that increasing the height of the fill reduced the relative amount of organic and inorganic pollutants leached from the solid waste. The longer distance that the organic pollutants had to travel through the refuse enabled the bacteria present in the fill to partially remove the degradable organics, and the percentage of organics with a biochemical oxygen demand (BOD) extracted per dry weight of solid waste decreased from 5.2% to 2.6% when the height of the fill was increased from 0.76 m to 3.05 m. A similar pattern was noted for most inorganic pollutants. Iron, however, showed a deviation from this, since the amount extracted per dry weight of solid waste increased by 30% when the height of the fill increased from 0.76 m to 3.05 m. As this pattern is the inverse of that observed for the degradable organics the relative increase in iron content may indicate that a substantial portion is chelated by refractory organics, that are formed by bacteria after the removal of more biodegradable organics.

More recent leachate studies have included several other metals in their measurements. Fungaroli and Steiner (1971) showed that zinc (Zn) is generally present at a concentration one-tenth of that of iron in leachate obtained from a lysimeter. They also showed that the iron concentration was proportional to the volume of the generated leachate indicating that the infiltration flow has to be sufficiently high to leach the iron. Since the higher leachate production corresponded to a lower pH, caused by a higher free volatile fatty acid content, the variation in the iron content may also be the result of its pH dependent solubility. The Zn increase lagged behind that of the iron content, indicating that the former element was only leached after the solid waste degradation had proceeded substantially. The nickel (Ni) concentration generally ranged from 0.2 to 0.3 mg/l and its variation with time was generally the opposite of the iron content.

Lower heavy metal concentrations were measured in leachate from actual landfills as compared to leachate generated from lysimeters filled with solid waste. A survey by Hughes *et al.* (1971) of several full-scale landfills in Illinois showed that the iron and zinc content were generally less than 100 mg/l and 5 mg/l, respectively. Several heavy metals such as copper (Cu) and cadmium (Cd) were usually below their detectable limits.

The County of Sonoma study (1973) measured the heavy metals content in leachate from a control pilot landfill and a pilot landfill in which the collected leachate was reapplied onto the fill, resulting in a higher moisture content of the solid waste. A calculation based on the reported data showed that the higher moisture content did not have a major effect on the composition of the inorganic salts. Using the chloride concentration as the base for comparison it was computed that the ratio of Ca/Cl was 1.17 for the control fill and 1.10 for the recirculation fill. The relative zinc content was slightly lower for the control fill since the Zn/Cl ratios were 0.014 and 0.022, respectively. The relative iron content, however, was approximately 10 times higher for the control fill based on the Fe/Cl ratios of 1.72 and 0.18, respectively. The relatively higher iron content may be the result of the higher chelation of the organics present in the control fill since the relative organic matter content based on the chemical oxygen demand (COD)/chloride (Cl) ratio was 86 for the control fill and 29 for the recirculation fill.

Interferences in the Heavy Metal Determination

Many techniques are presently available to determine heavy metals in aquatic systems and three basic methods can be distinguished: electronic transition methods (molecular absorption spectrometry, molecular fluorescence spectrometry, atomic absorption spectroscopy, atomic fluorescence spectrometry, atomic emission spectroscopy, X-ray emission spectroscopy), neutron activation methods, spark source mass spectrometry, gas chromatography and electrochemical techniques (Minear and Murray, 1973). Many studies in the area of water pollution use the flame atomic absorption spectroscopy and this method was also employed in the present study. The method involves the atomization of the sample by flame and the absorption of radiation at the specific wavelength of the metal. The radiation source used is a hollow cathode lamp consisting of a cathode made of the same element as the metal under analysis (Willard *et al.*, 1965).

Leachate samples will present numerous difficulties in flame atomic absorption analysis for heavy metals such as Cr, Ni, Fe, Pb, Cu, and Zn, due to the presence of complex matrices. Similar interferences have been encountered in the analysis of heavy metals in sea water which has an average salt concentration of 35,000 mg/l (Horne, 1969). In comparison, leachate samples can have a fixed solids content of up to 23,000 mg/l (Chian and DeWalle, 1976).

Interference effects in flame atomic absorption can be classified into three main groups: 1) spectral, 2) non-atomic absorption, and 3) chemical (Dean, 1960).

Spectral or line interference can often be avoided by judicious choice of an analyte resonance line and by using a narrow band-width as close as possible to the width of the selected line emitted by the hollow cathode tube. Kahn and Manning (1972) showed that absorption band-widths in a flame can be as small as 0.03 Å. The best resolution obtained in commercially available units, however, is only 0.3 Å, while most units have monochromatic spectral band-widths of 2 Å or more.

Non-atomic absorption can result from the flame itself, molecular absorption from the elements in the samples or light scattering caused by unburned particles in the optical path. These effects are generally most pronounced at shorter wave lengths in the far UV portion of the spectrum. This background absorption effect can be eliminated by using a two-line method, i.e., subtraction of the absorption at a non absorbing line close to the analyte line, and by using a deuterium background correction or employing a hydrogen hollow cathode lamp.

The last type of interference effects, which are element specific, are expected to cause complications in leachate analyses. Specific chemical interferences have long been known in atomic absorption spectroscopy (Table 14). Alkali and alkaline earth elements such as Na and K are subject to a wide range of interferences, primarily because they ionize in the flame and are thus unavailable for atomic absorption of their resonance line energy emitted by the hollow cathode lamp (Johnson and Schrank, 1964). This situation can be corrected, however, by the addition of a more easily ionized element such as Cs at a concentration level considerably higher than that of the analyte atom. Ca and Mg present a more serious problem since they are interfered with by a number of common cations and anions, such as $SO_4^=$, $PO_4^{\equiv}$, $CO_3^=$, HCO_3^-, Na^+, K^+, and Al^{+++} (Ramakrishna, 1968). An inorganic releasing agent, lanthanum, for instance, has been found effective in suppressing interferences and prevent formation of oxides in the flame (Adams, 1966).

Table 14

Chemical Interferences Observed in the Metal Determination by Flame Atomic Absorption Spectroscopy (Hermann, 1963)

Interferent	Analyte										
	Ca	Cd****	Cr	Cu	Fe	K****	Mg	Na	Ni	Pb****	Zn*****
Al	d*		d	e			d	d			
As	d										
B	e**							d			
Ba	d			d				eed			
Be								d			
Br								d			
$CO_3^=$											
Ca							de	eed			
Cd	e			d				d			
Cl	dde***		e	dde			d	d			
ClO_4	eed		d	ed			e	d			
Co				e				d	e		
Cr	d			d				dde	e		
Cs				e				d			
Cu								d			
Fe	d		d	e			e	ed	e		
IO_4	d		d	e			e	ed	e		
K	e			e			e	eed			
HCO_3^-											
Li	d							dde			
Mg	ed			edd				d			
Mn	d			d			e	dde	e		
Mo								d			
NH_4					d						
NO_3	d		d	de			d	d			
Na	d			d			e				
NaCl					d						
Ni				e				e			
P	dde		e	e			dde	d			
PO_4					d						
Pb								d			
Rb								d			
S	d		e	e					e		
Si	d				d						
Sn				dee							
Sr	e							e			
SO_4					d						
Te	d										
Ti	d						d	d			
TiF_6	d										
W								d			
Zn	d			e				d			
Zr	d										
Organics	eed		eed	eed			eed	eed	eed		
Citrate					d						

* d = depression

** e = enhancement

*** dde = depression at low and intermediate concentration and enhancement at high concentrations

****no information yet available

Detailed specific interferences for heavy metals such as Fe, Cu, Cr, Ni, Sr, and Al are given by Herrmann (1963) and Yanagisawa *et al.* (1970). Cr is the most susceptible to interferences. The iron determination has been shown to be affected by silicate, sulphate, phosphate, and ammonium ions (Roos and Price, 1971).

Nonspecific interferences such as acid[illegible] salts hinder the evaporation of the solution or the solvent and increase the droplet size resulting in a diminished quantity of fine aerosol reaching the burner; surface tension and viscosity can have a similar effect. Gilbert (1962) reviewed the theory of chemical interferences and stated that the situation is complex and not well understood. Since the chemical interference generally results in a depression of the absorbance, erroneously low metal concentrations would be obtained if this factor is not evaluted.

Interaction of Heavy Metals and Organic Matter

Much controvorsy exists as to whether the heavy metals in aquatic systems exist in solution or are chelated by organic matter or adsorbed to suspended particles. Stumm and Bilinsky (1973) calculated that trace metal complexes are unimportant because of the large amount of cumpeting Ca^{++} and Mg^{++} ions. Siegel (1971) also cuncluded that a chelator has an impact only when the stability constant with the trace metal is about six times higher than the Ca^{++} and Mg^{++} complex. Experimentcl evidence by other researchers shows, however, that the heavy metals Pb and Co could be complexod significantly with humic matter (Barsdate and Matson, 1966). They revealed, for example, that Co, Zn and Mn dissolved in strongly colored water could not be removed by dialysis. Slowey *et al.* (1967) found that 8-50 percent of the copper in Gulf of Mexico water could be extracted into chloroform, and that this was the result of ligands present in the water.

Studies by Hodgson *et al.* (1966) and Geering *et al.* (1969) showed that 98-99 percent of the Cu, 84-89 percent of the Mn, and up to 75 percent of the Zn in soil solutions occurred in organic complexes. In a further study these authors concluded that the micro nutrients were largely associated with low molecular weight dialyzable organic constituents. Schnitzer and Kahn (1972) reported that the major chelation of the heavy metals involved the combined presence of phenolic and carboxylic groups and that minor chelation took place through the carboxyl group alone. Broadbent and Bradford (1952) also showed that carboxyl and phenolic groups, attached to heterocyclic compounds, are important functional groups for the Cu binding capacity. Broadbent (1951) further concluded that while the carboxyl groups complexed both Cu and Ca, other functional groups reacted selectively with Cu to the exclusion of Ca.

Shapiro (1964) showed that fulvic acids present in fresh water are associated with iron. Christman (1970) presented evidence that the majority of the iron was complexed and associated with all size fractions of the organic matter. A strong chelation agent was able to remove some of the iron but it was least efficient in removing the iron from the fraction with a molecular weight larger than 50,000. Considerable amounts of heavy metals can also be expected to adsorb to the ferric hydroxide colloids (Barsdale and Matson, 1966).

In the case of leachate, the organic complexing is most likely governed by the Fe, Ca, and Mg concentrations which are competing with the heavy metals for the ligand sites. If complexing occurs, it can be an important mechanism in the transport of heavy metals in groundwater. Its attenuation would then be govered less by the solubility of their hydroxides but by the ability of the soil materials to remove the cheland.

The above survey showed that only a limited number of studies have investigated the presence of heavy metals in landfill leachate. None of the studies investigated whether the atomic absorption spectroscopy used to measure the heavy metal content was interfered by matrix effects of the high salt concentration in the leachate. Based on the results listed in Table 14 such is indeed expected. Some chelation of the heavy metals is also expected, the magnitude of which would depend upon the concentration of Ca and Mg competing for the absorption sites. It was therefore the purpose of this study to determine the actual concentration of heavy metals in leachate and to measure the magnitude of the matrix effect of the salts. The second part of the study measured the interaction of the heavy metals with the organic matter and determined the molecular weight fractions that showed the strongest chelation with the heavy metals.

MATERIALS AND METHODS

The leachate samples were collected under strictly anaerobic conditions followed by ultracentrifugation and 0.45 μ membrane filtration to remove any solids present in the sample. Prior to flame atomic absorption measurements, the organic matter in the sample was removed using a rigorous digestion procedure, which also served to eliminate any metal hydroxydes. The procedures used for pretreating the leachate sample are those recommended by the US-EPA (1971) and 50 mℓ of sample was gently digested with two portions of 3 mℓ concentrated HNO_3 whereafter 3 mℓ of 1:1 HCl was added. Silicates that had formed on the bottom of the beaker were removed by filtration. The atomic absorption unit used for the present study was a Beckman Model 485 (Fillerton, Cal.).

It was found that the high salt content in leachate greatly limited direct aspiration of the sample. A high solids burner was tested, but it did not have the desired sensitivity. Chelation with ammonium pyrrolidine dithiocarbamate (APDC) and extraction into methylisobutyl ketone (MIBK) was evaluated next. However, the high salt content caused the formation of an intractable emulsion which likewise would not aspirate.

The final method used in this study was to dilute the leachate and to use the standard addition technique (Christian, 1969), in which the sample is spiked with increasing amounts of the analyte. This method can only be used when the absorbance is linear over the concentration range involved, while the relative magnitude of the chemical interference should also be constant within that range. The non atomic absorption will have to be measured at each standard addition since otherwise the calculated concentration would be greater than it actually is.

The determination of the alkali and alkaline earth metals did not give any complications. It was found satisfactory to add 10,000 mg/ℓ La to the leachate samples to suppress the ionization of Ca and Mg. To each sample analyzed for Na and K, 1000 mg/ℓ of Cs was added to suppress the ionization of the analyte atom. The elements Cd, Cr, Cu, Ni, Pb and Zn were determined using the standard addition method.

Leachate samples were collected from 12 landfills of various ages located in different regions of the United States and representing different climatic environments (Chian and DeWalle, 1976). The strength of the organic pollutants varied from 81 mg/ℓ to 71,080 mg/ℓ COD, while the inorganics varied between 812 mg/ℓ to 22,895 mg/ℓ fixed solids. As a result of these various strengths the magnitude of the depression also varied considerably. In order to further evaluate some of the interferences and the applicability of the standard addition method to leachate analysis, four synthetic samples were devised containing 1 mg/ℓ of each of the heavy metals. The composition of the major ions in these synthetic samples as listed in Table 15 were chosen to resemble actual concentrations that were found in analyzing the various leachates (Chian and DeWalle, 1976). The first two samples contained a low total dissolved solids (TDS) content while the last two samples had a salt content approximately five times higher. The first and the third sample had a relatively high sulfate content while the second and the fourth has a high chloride concentration.

The next phase of the study consisted of fractionating the organic matter in a leachate sample obtained from a lysimeter installed at the University of Illinois. The organics were separated according to their molecular weight using membrane ultrafiltration techniques and gel permeation chromatography (DeWalle and Chian, 1974). The ultrafiltration membranes used were UM05, UM10 and XM100A having nominal molecular weight cutoffs of 500, 10,000 and 100,000 (Amicon, Lexington, Mass.), respectively. The different ultrafiltration membrane fractions were characterized by total organic carbon (TOC), chemical oxygen demand (COD), carbohydrates (anthrone test), proteins (ninhydrin test after 24 hr acid digestion), carboxyl groups (hydroxyl amine test), carbonyl groups (2,4 dinitrophenyl

Table 15

Synthetic Leachate Samples Used to Evaluate Matrix Interferences in the Heavy Metal Determination Containing 1 mg/l of Cr, Ni, Cu, Pb and Cd.(a)

	Sample Number			
Elements	1 (mg/l)	2 (mg/l)	3 (mg/l)	4 (mg/l)
Cl	50	1000	50	1000
Na	32	640	32	640
SO_4	1000	50	1000	50
K	670	34	670	34
Ca (as $CaCO_3$)	100	100	2000	2000
Mg (as $Mg[C_2H_3O_2]\cdot 4H_2O$)	30	30	500	500
Fe^{3+}	50	50	500	500
TDS	2246	2218	10482	10454

(a) All samples were diluted 1:2 when the standard additions were added, resulting in concentrations half of those listed above hydrazine test), and aromatic hydroxyl groups (Folin-Denis test). After the heavy metal distribution in each of the molecular weight fractions was established, additional leachate samples previously analyzed for their total heavy metal content, were fractionated with a 10,000 MW ultrafiltration membrane whereafter the heavy metals in each molecular weight fraction were determined.

RESULTS AND DISCUSSION

The results of the standard addition technique in a 1:8 diluted leachate sample are shown in Figure 1. The leachate sample was obtained from the experimental US-EPA pilot landfill at the Boone County Field Site, Ky and referred to as the Cincinnati sample (SHWRL, 1973). The sample had an initial COD of 45,750 mg/ℓ and a fixed solids content (FS) of 13,603 mg/ℓ. The depression caused by the constituents in the leachate ranged from 40 percent for Cr to 5 percent for Cd (Figure 22). The depression was calculated as the tangent of the function between the amount recovered and amount added, divided by the tangent of the 100% recovery function. When, therefore, a heavy metal determination is made without application of the standard addition the measurement can be as much as 40 percent lower than the actual value. When the determination is made on a leachate sample without prior dilution, the matrix of the sample may even result in a higher depression and further underestimate the actual value.

In addition to the Cincinnati sample (CINC) two samples collected from a milled uncovered (MUNC) and an unmilled covered (UMC) solid waste lysimeter in Madison, Wisconsin (Reinhardt and Ham, 1973) also experienced considerable depression. Two samples obtained from a pilot solid waste lysimeter (UI 2/1 and UI 9/21) at the University of Illinois (Chian and DeWalle, 1976) were also evaluated. The percent depression of these five samples was conveniently plotted against the wavelength at which each element was measured (Figure 2a). The results of the five samples are qualitatively similar as the magnitude of the depression decreased from Cu to Cr, Ni, Pb, Cd and Zn respectively. Several factors may have caused the depression in the recovery of metal analysis.

Comparison of the percent depression with the salt concentration in the diluted leachate samples indicated that some relation existed. For example, the highest depressions for Cr were observed with the samples

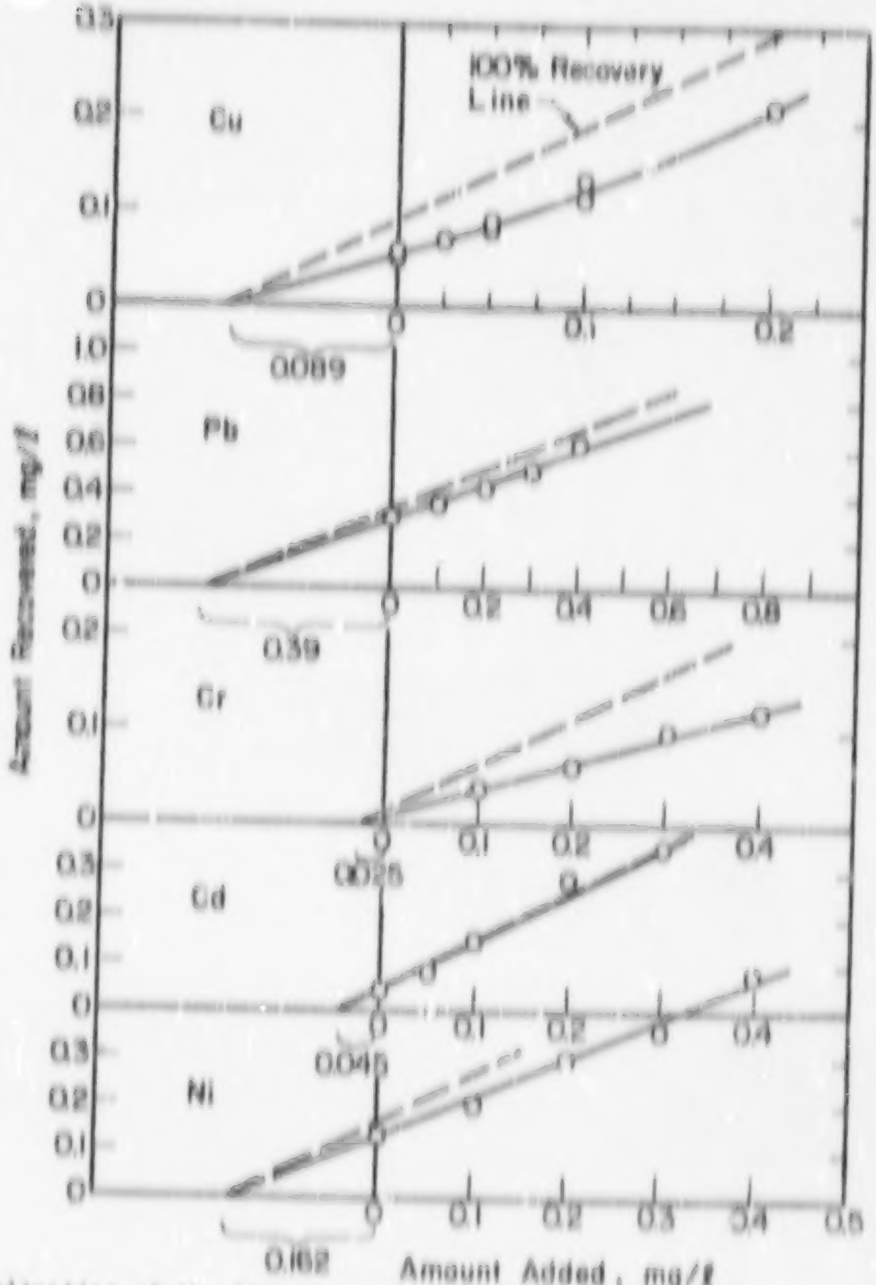

Figure 22. Application of the Standard Addition Method to Determine the Actual Heavy Metal Concentration in the Cincinnati Field Site Leachate Sample

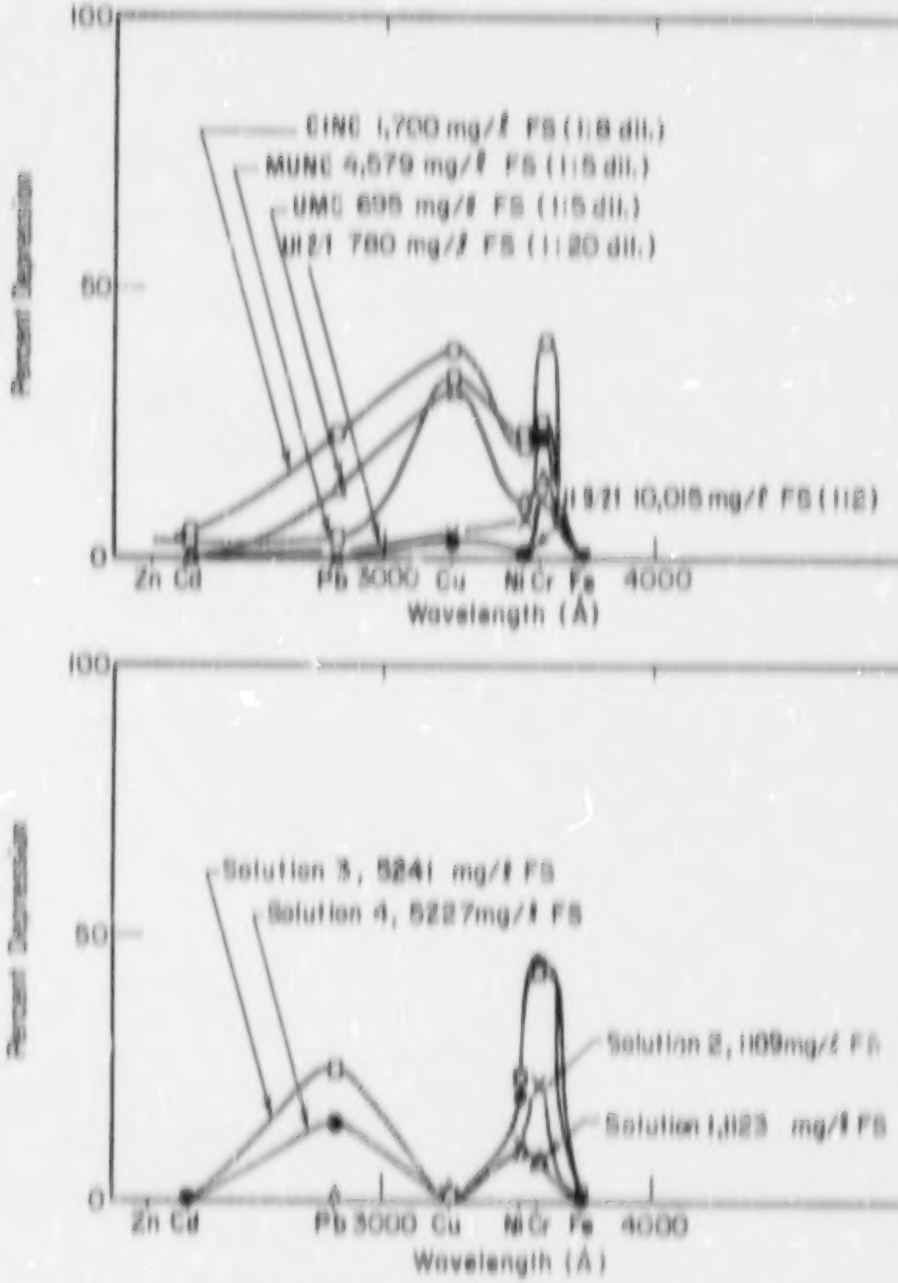

Figure 21. Percentage Depression in the Heavy Metal Determinations for Leachate Samples (a) and Synthetic Salt Solutions (b).

having salt contents of 1700 mg/ℓ, 4570 mg/ℓ and 10,015 mg/ℓ while the lowest depression occurred in the samples with a salt content of 695 and 780 mg/ℓ. The relationship between depression and salt content, however, is certainly not linear for Cr. The Cincinnati sample, for example, with a salt content of 1700 mg/ℓ resulted in a depression of 40 percent, while the University of Illinois sample (UI 9/21) having a salt content of 10,015 mg/ℓ in the 1:2 dilution, gave a depression of only 23 percent. Comparison of the depression of the heavy metal analysis of leachate samples with those of the synthetic salt solutions (Figure 2b) confirmed the effect of the concentration of inorganic salts on the Cr determination. The highest depression was indeed found for solutions 3 and 4 with a TDS of 6,900 mg/ℓ and 5,021 mg/ℓ, respectively. In addition to the absolute salt content, the relative salt concentration also seems to be an important parameter. For example, the ratio of fixed solids/Cr in the CINC, MWRC, UMC and UI (2/1) samples decreased from 68,015 to 45,790, 19,306 and 866, respectively, corresponding with a percentage depression which decreased from 40% to 25%, 14% and 11%, respectively. The results with the synthetic leachate samples also show that the chloride concentrations have a pronounced effect on the depression of the absorbance since the 1000 mg/ℓ Cl^- in solution 2 resulted in a depression of 22 percent, while the 50 mg/ℓ in solution 1 caused a depression of only 6 percent; both solutions have a almost identical salt concentration. Similar conclusions with regard to the chloride effect can be drawn from the chemical analysis of the tested leachate samples as listed in Table 16. This, however, is contrary to the results presented in Table 14, which showed that chloride actually enhances the Cr absorbance.

Although most of the chemical interferences in the Ni determination tend to enhance the absorbance (Table 14), the constituents present in leachate cause an apparent depression (Figure 23). However, the depression for Ni in leachate samples and the synthetic salt samples is generally lower than that observed for Cr. When the percentage depression was correlated with different factors that could cause such depression, the best correlation

Table 16

Chemical Characteristics of Leachate Samples in Which the Heavy Metals were Tested by the Standard Addition Method

Parameter (mg/l)	Source University of Illinois (UI 2/1)	Madison (MUNC)	Madison (UMC)	Cincinnati (CIN)
COD	49,300	71,680	16,580	45,750
TOC	17,060	27,700	5,906	13,840
BOD	24,700	57,000	9,960	22,000
Acetic acid	4,370	6,690	610	1,340
Propionic acid	1,050	3,180	255	660
Isobutyric acid	570	360	31	340
Butyric acid	5,620	9,270	480	1,700
Isovaleric acid	1,220	770	40	520
Valeric acid	960	1,260	265	460
Caproic acid	2,400	1,920	565	1,090
Bicarbonate alkalinity	668	459	284	175
pH (-)	5.63	5.97	5.17	5.25
Cond (µmho/cm)	13,700	16,800	5,420	9,450
ORP (mv)	-60	-132	-220	*
Turb (ZTu)	75	80	270	71
Suspended Solids (SS)	139	202	192	8.9
Fixed Suspended Solids (FSS)	92.5	167	110	7.5
Total Solids (TS)	33,989	55,348	7,930	32,145
Fixed Solids (FS	15,586	22,348	3,475	13,603
Org-N	544.7	945	78.5	31
NH_4-N	392.6	1,028	347.4	247.7
NO_3-N	0.5	10.25	4.25	9.8
NO_2-N	BDL**	0.04	0.04	0.19
Tot-P	21.5	98	85	31.6
Ortho-P	5.5	29	85	28
SO_4	1,110	1,558	77	909
Cl	1,480	2,467	474	2,096

As a result of the standard addition the samples were diluted 1:20, 1:5, 1:5, and 1:8, respectively.

was obtained with the fixed solids content in the diluted leachate sample. The result of the synthetic sample analysis indicated that neither sulfates nor chlorides had any effect. High correlations were noted for Ca and Fe possibly indicating that multivalent cations are the main cause of the observed depression.

The results for Cu show no interference in the synthetic salt solution while the depression was relatively large in all leachate samples except for the two UI samples. A main difference between the leachate samples and the synthetic salt solutions is that only the former contain nitrogenous substances, such as ammonia, nitrate and nitrite, and phosphates. Of these, the relative phosphate concentrations showed the best correlation with the percent depression. The parameter that showed the best correlation with the percent depression, however, was the relative zinc concentration, indicating that the heavy metals can have an effect on each other.

Evaluation of the interference for Pb indicates that a high salt content increases the depression. At a high relative salt content, however, the depression tended to decrease again. The results with the simulated leachate samples indicates that a high sulfate content also increases the depression. The determination of Zn and Cd is generally not strongly interfered with by matrices present in leachate samples and the measurement can be made on a sample without using the standard addition technique.

The metals present in relatively high concentrations in leachate such as Ca, Mg, and Fe were next evaluated. When Fe was added at a concentration of 50 mg/ℓ and 500 mg/ℓ to the four synthetic salt solutions which was subsequently diluted 1:10 and 1:100 to result in a final concentration of 5 mg/ℓ no depression was observed; instead a slight enhancement in all four samples was noted. The synthetic salt solutions were also used to determine the recovery of the Ca ion. Solutions 1 and 2 were diluted 1:20 and solutions 3 and 4 diluted 1:400 to result in a final Ca concentration of 5 mg/ℓ. The measurement of the Ca showed that less than 100 percent was recovered. The depressions in the four salt solutions were

12, 11, 12 and 15 percent, respectively. The addition of 10,000 mg/l La, however, was found to eliminate the interfering effect. The effect of omitting the La addition on the Mg determination was less pronounced and the Mg values in the absence of the La were even slightly higher than in the samples to which La was added. The Mg determinations also showed that the salt solution with the highest salt content showed the highest enhancement effect.

Analysis of metals in less polluted leachate samples collected from additional landfills described by Chian and DeWalle (1976) was not interfered with by the matrix of the sample since the relative or absolute salt content was sufficiently low at the dilutions used. The results of the flame atomic absorption measurements for each of the 12 leachate samples are listed in Table 17. The values for the different landfills show a gradual decrease corresponding with an increasing age of the landfill. Such a decrease is expected since continuous leaching will remove the metals from the refuse, and since the leachate experiences a gradual pH increase with the increasing age of the fill that decreases the solubility of each of the metals. Although the trend of decreasing concentrations with increasing age is quite noticeable, considerable variation does occur at a specific landfill age. To eliminate these variations the relative concentration of the metal was calculated with respect to the other inorganic salts as measured as fixed solids (FS). These ratios were then plotted against the age of the landfill (Figure 24). The relative Ca, Fe and Zn concentrations show a gradual decrease. As the Ca is the most soluble of the three metals shown, its decrease with age is the least rapid. Its decrease is probably the result of its percipitation as carbonate salt. The decrease of the relative Fe and Zn concentrations follows a pattern inverse to that of the oxidation reduction potential. Their relative concentrations do not seem affected by the organic matter decrease. Their more pronounced decrease after a five year period is more abrupt than noted for the organic matter decrease, which may indicate that most of the metals are not affected and thus not chelated by the organics in leachate.

Table 17

Metal Concentrations in Leachate Samples Collected from Landfills Having Different Ages

Parameter	Source					
	University of Illinois (UI 2/1)	U. of Wisconsin Madison (MUNC)	U of Wisconsin Madison (UMC)	Sonoma, Ca Control (SONCON)	Sonoma, Ca Recirculation (SONREC)	SHWRL Cincinnati, OH (CIN)
Ca	3750	3900	572	1030	1220	2325
Mg	650	1140	220	515	750	530
Fe	2200	1046	91	301	540	735
Na	1360	1580	330	240	675	750
K	1140	2300	900	74	300	860
Zn	104	170	13	22	15	52
Cd	0.1	0.375	0.05	0.02	0.008	0.36
Pb	1.25	1.65	0.80	1.1	0.48	3.12
Cu	0.40	0.75	0.65	1.0	0.1	0.71
Ni	13	2.6	0.23	0.5	0.6	1.3
Cr	18	0.5	0.18	0.11	0.15	0.2
Age of Fill (yr)	0.25	0.33	0.33	1.64	1.64	2.3
	GIT Atlanta Control (ATLCON)	GIT Atlanta Recirculation (ATLREC)	College Park, PA (PACP-67)	Kennet Squire, PA (PHKS-12)	W'nnetka, Illinois (LW-17)	Winnetka, Illinois (LW-58)
Ca	520	133	215	330	254	76
Mg	52.5	35	51.5	85	81	186
Fe	810	0.5	560	330	1.5	4
Na	75	44	37.5	113	91	370
K	620	147	35	70	39	450
Zn	74	0.05	6.5	2.5	0.16	1.02
Cd	17	0.03	0.01	0.005	0.003	0.003
Pb	1.16	0.10	0.21	0.2	0.48	0.54
Cu	0.48	0.01	0.15	0.21	0.10	0.32
Ni	0.4	0.05	0.09	0.32	0.08	0.12
Cr	0.03	0.03	0.015	0.095	0.04	0.04
Age of Fill (yr)	2.25	2.25	5.3	6.75	4.83	16

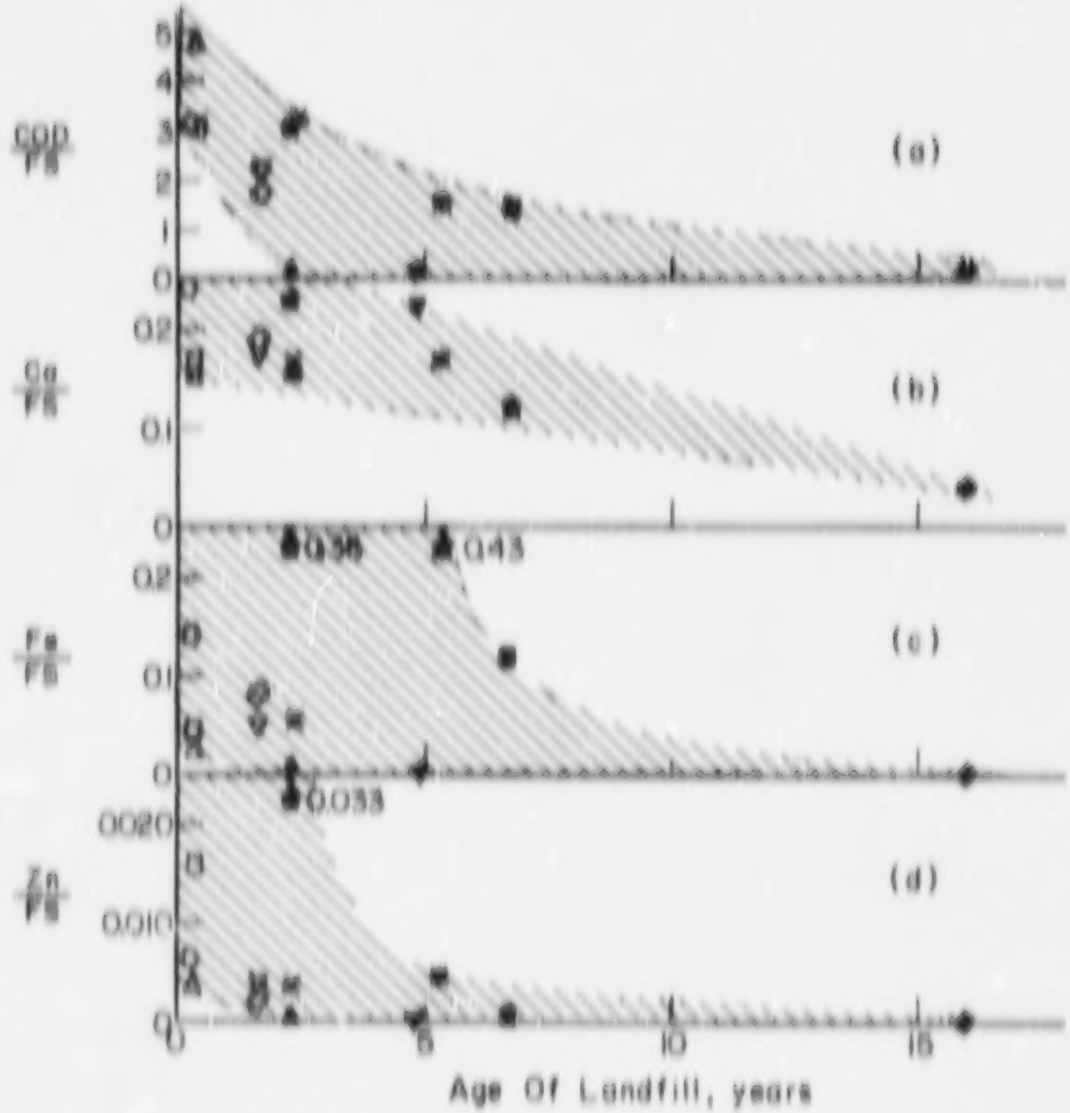

Figure 24. Decrease of COD, Ca, Fe and Zn Relative to the Salt Content of Leachate Samples with Increasing Age of Landfills

To study the interaction of heavy metals with the organic matter in the leachate sample an extensive fractionation was made of the different molecular weight fractions followed by their organic analysis. The first fractionation conducted on the leachate sample collected from the University of Illinois lysimeter using a 500 MW UF membrane showed that the majority of the organics had relative low molecular weights since 72.8 percent of the TOC was recovered in the permeate. The majority of the metals were also recovered in the permeate and only 2.4 percent of the Mg and 2.5 percent of the Ca were measured in the 500 MW UF retentate, while a slightly larger percentage (5.5 percent) of Zn was retained. The majority of the iron on the other hand was recovered in the UF retentate and only 41 percent was detected in the permeate.

The 500 MW UF retentate was then diluted to its original concentration and subsequently passed through a 10,000 MW UF membrane which now retained 21.8 percent of the original TOC. When the diluted 10,000 MW UF retentate was subsequently passed through a 100,000 MW UF membrane the percentage TOC decreased further to 11.1 percent of the original TOC. The results of the organic analysis for different classes of compounds and functional groups of the 4 membrane fractions showed a decreasing carbohydrate content with decreasing molecular weight. The proteins followed a trend similar to that of the carbohydrates in the fractions larger than 500 MW. A surprisingly high content was also found in the 500 MW UF permeate indicating the presence of amino acids. The carboxyl group analysis showed that the carboxyl group density increases with decreasing molecular weight. The highest percentage was present in the 500 MW UF permeate due to the presence of the free volatile fatty acids. Gas chromatographic analysis showed free volatile fatty acids, predominantly acetic and butyric acid. The carbon of these acids comprised 78 percent of the TOC of the 500 MW UF permeate or 49 percent of the initial TOC of the leachate sample.

Each of the fractions that were analyzed for functional groups were also subjected to heavy metal analysis. The results in Figure 25 show that

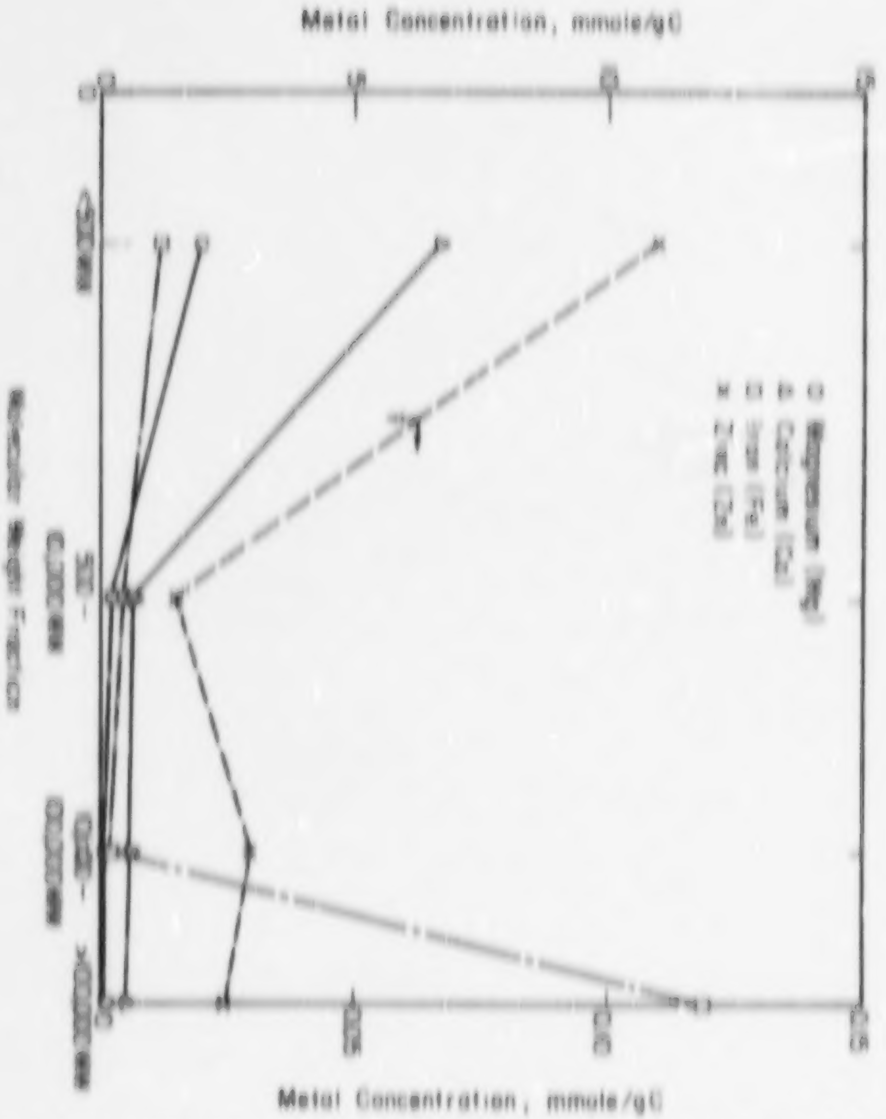

Figure 25. Relative Distribution of Metals in Each of the Different Molecular Weight Fractions as Obtained by Membrane Ultrafiltration

the relative concentration of metals (expressed as mMole/g organic carbon generally decreases with increasing molecular weight, a trend parallel to that observed for the carboxyl group. Calcium, magnesium and iron were the metals retained in highest quantity followed by zinc. These results tend to confirm the model studies of Stumm and Bilinsky (1973) who calculated that high concentrations of Ca and Mg will prevent the chelation of substantial amounts of heavy metals. The results obtained for iron in the present study forms an exception in this respect, since it has a low relative concentration in the 500 MW UF permeate and high relative concentrations in the 100,000 MW retentate. Similarly Shapiro (1964) and Christman (1970) reported relatively high amounts of iron associated with the high molecular weight organics as obtained by gel permeation chromatography.

The sum of the different metals in the 500-10,000 MW fraction represents 1.30 mMole/g C and assuming that the iron is also in the divalent form, this equals 2.6 meq/g organic carbon. The carboxyl group density as determined by titration and confirmed with the hydroxyl amine test is equal to 10.1 meq/g C, indicating that approximately 26 percent of the carboxyl groups have divalent metals as counter ions. As a result of this divalency, these metals may well be strongly retained or chelated by the organic matter. The other carboxyl groups may not have been dissociated at the pH of 6.5, i.e. the pH at which the fractionation was performed. An undetermined amount of K and Na ions will also be present as counter ions in each of the molecular weight fractions. However these monovalent cations are less preferred than divalent cations and are therefore not expected to be present in large quantities. The metals present in the 10,000-100,000 MW UF fraction are equal to 1.75 meq/g organic carbon which also represents approximately one fourth of the carboxyl content of 6.6 meq/g C. Due to the high iron content the metals would represent 377 percent of the carboxyl group content in the 100,000 MW UF retentate, thus indicating that the majority of the iron is present as colloids, possibly ferric hydroxides. The formation of such colloids was shown to occur directly after collection of the leachate sample as a result of a noticeable increase of the oxidation reduction potential despite the strict anaerobic collection procedures (Chian and DeWalle, 1976). The Zn shows an interesting pattern in that its relative concentration in the larger than 500 MW fractions tends to be the opposite of that of the iron indicating that some competition for chelation sites may occur.

The last phase of the study consisted of fractionating 8 of the 12 collected leachate samples according to their molecular weight using a 10,000 MW UF membrane to determine the amount of heavy metals present in the UF retentate. The 10,000 MW UF membrane was selected since it was best suited to obtain the high molecular weight fraction. The percentage of the organic matter retained was then related to the percentage of each of metals retained. The results in Figure 26 show that both the percentage Ca and Fe retained with the 10,000 MW UF membrane increases with increasing magnitude of the high molecular weight organic fraction. The calcium content showed a larger than proportional increase with increasing magnitude of the high molecular weight fractions, while the retained iron increased proportional to the high molecular weight organic fraction. The observed increase in the amount of calcium retained with increasing size of the high molecular weight fraction may be due to chelation of the calcium by the carboxyl - and aromatic hydroxyl groups of the high molecular weight organic fraction. Its more than proportional increase may indicate that the amount of functional groups associated with this organic fraction increases with increasing concentration of this fraction. As most of the iron is present as ferric hydroxide colloids, the increase in amount retained with increasing size of the high molecular weight fraction indicates that both parameters show a significant interaction. The high molecular weight organics could be adsorbed onto the colloidal particles, resulting in a larger complex which is therefore better retained with the 10,000 MW UF membrane. When both the Ca and Fe were plotted in the form of a Freundlich isotherm with the metal content in the 10,000 MW retentate expressed as meq/g organic carbon and the concentration in the permeate as

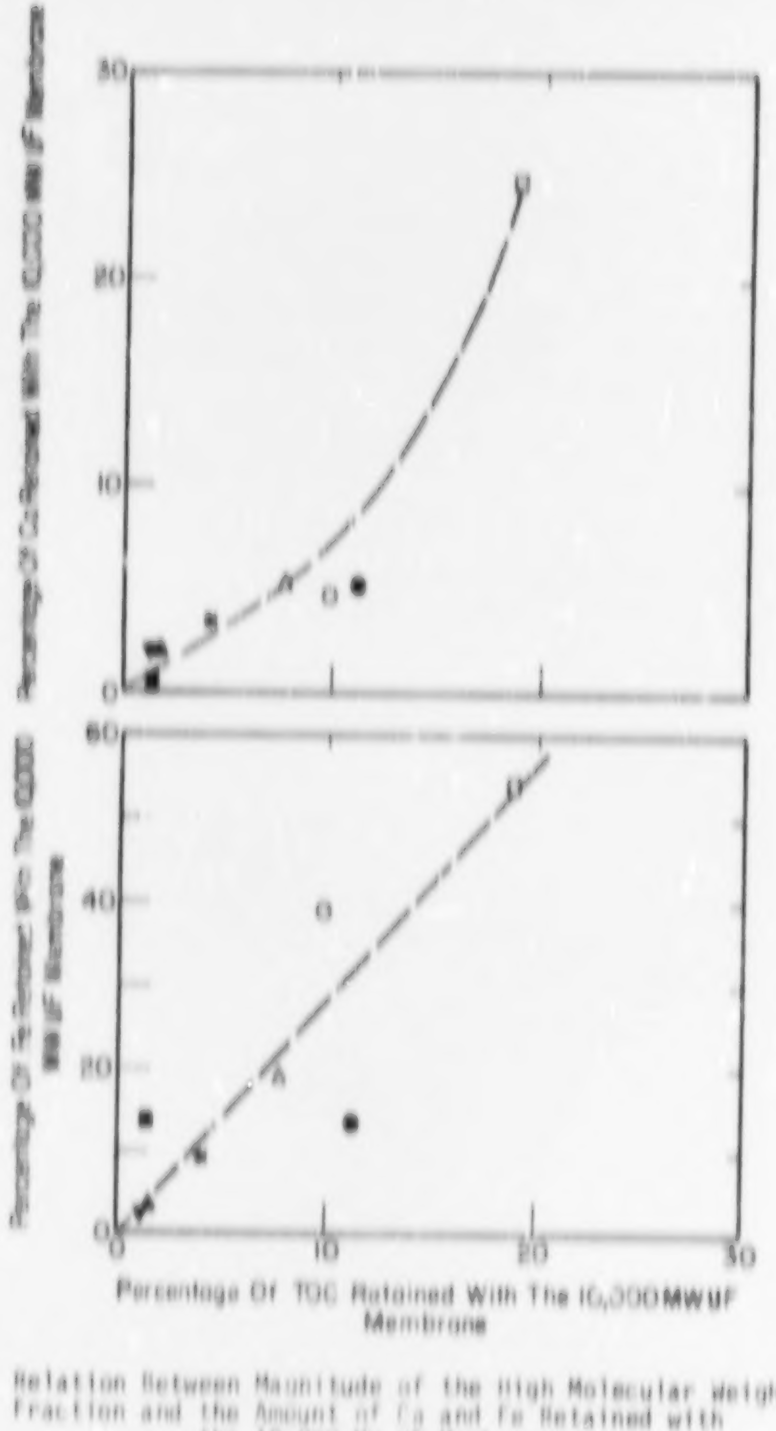

Figure 26. Relation Between Magnitude of the High Molecular Weight Organic Fraction and the Amount of Ca and Fe Retained with the 10,000 MW UF Membrane

equilibrium concentration, the curve for the Ca ion showed a slight decrease while the Fe showed an increase in amount retained with increasing equilibrium concentration. Since the magnitude of the total iron concentration parallels that of the calcium concentration, this may indicate that the iron is able to partially displace the Ca in the high molecular weight fraction at increasing iron concentration.

The results for Cu (Figure 27) show that the percentage retained gradually decreases with increasing magnitude of the high molecular weight fraction. Only one sample having a substantial concentration of high molecular organics deviated from this trend as it also showed a large amount of copper retained. The Zn showed a similar result as the Cu for small sizes of the high molecular weight fraction. At high percentages of the TOC retained with the 10,000 MW UF membrane the percentage Zn retained showed an increase. When the data were plotted as a Freundlich isotherm, the amount of Cu adsorbed per gram organic carbon showed a significant decrease at increasing equilibrium concentrations. The amount of Zn adsorbed per gram organic carbon, however, experienced a gradual increase at higher equilibrium concentrations. The increase was lower than that observed for iron possibly indicating iron is complexed preferentially to the zinc, which in turn is preferred to Ca and Cu at the concentrations studied. Since ion exchange mechanisms may also exert a considerable effect, a change in absolute concentration of each of the metals may change the preference of chelation as noted above. The increase of amount adsorbed at higher equilibrium concentrations for Zn and especially Fe may explain their generally high concentrations in leachate as compared to heavy metals of similar solubility such as Cd, Cu, Pb, Cr, and Ni.

The above results clearly indicate that the majority of the metals are not associated with organics having a molecular weight larger than 500. Interaction of the metals with the low molecular weight organics may well occur and has to be further evaluated. Such interaction, however, may be of an indirect nature since a high concentration of free volatile fatty

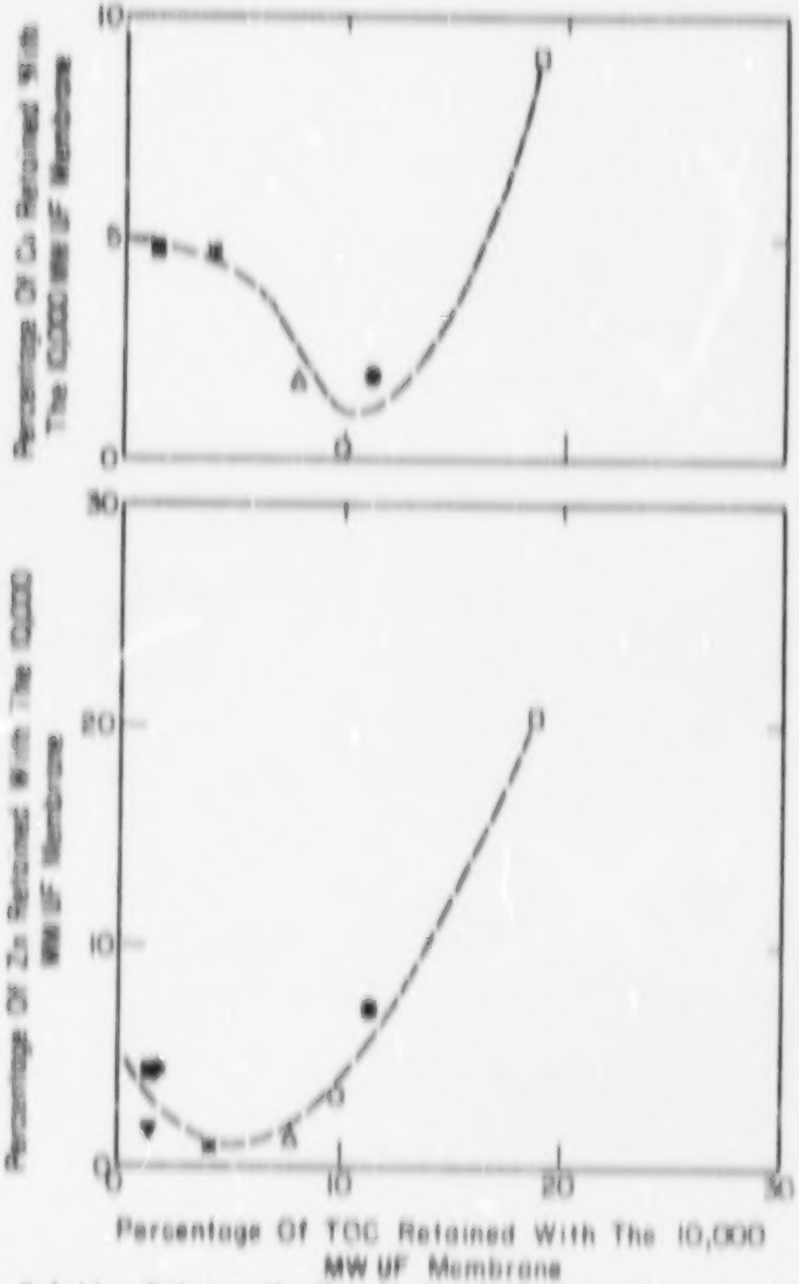

Figure 27. Relation Between the Magnitude of the High Molecular Weight Organic Fraction and the Amount of Cu and Zn Retained with the 10,000 MW UF Membrane

acids as found in high concentrations in the 500 MW UF permeate, will result in a lower pH of the solution and therefore a higher solubility of the metals.

Treatability studies with leachate in the second phase of the research project showed that aerobic biological systems generally resulted in a higher removal of metals as compared with anaerobic systems. While both anaerobic and aerobic treatment resulted in large reductions of the iron content, the reduction of Cr and Mg was larger in the aerobic treatment unit. No heavy metal toxicity has been observed in the aerobic units treating leachate but one such instance was observed in the anaerobic filter. The toxicity caused a gradual deterioration of the effluent COD of the unit and a gradual increase in Fe, Cu and Zn in the effluent. When the Fe and Cu concentration exceeded 3 mg/ℓ and 1 mg/ℓ, respectively, the methane bacteria were inhibited thus causing a sharp increase in free volatile fatty acids in the effluent and a decrease in gas production of the unit. Addition of sulfide to precipitate the heavy metals eliminated the toxicity. Sulfate in the leachate is reduced in the anaerobic filter to sulfide which then precipitates the heavy metals. The presence of 0.45 filterable ferric hydroxide colloids as found in the present study is apparently not presenting the removal of iron in the anaerobic filter; in effect it may enhance it. The possible chelation of zinc is apparently not effecting its removal as a zinc sulfide precipitate.

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VI

EVALUATION OF COLORIMETRIC TESTS USED FOR CHARACTERIZATION OF ORGANIC MATTER IN LEACHATE

CONCLUSIONS

The present study evaluated several colorimetric tests used to characterize functional groups and general classes of organics present in the different molecular weight fractions in leachate. Although most studies in the area of soil science use titrimetric methods for functional group analysis these methods were not applicable in the present study due to their low sensitivity. Colorimetric methods are preferred only when small amounts of organic matter are present. The hydroxyl amine test for measurement of carboxyl groups and free volatile fatty acids was evaluated extensively. The latter group of organics can also be measured using gas chromatography, employing m-cresol as an internal standard. The carbonyl groups were measured with the dinitrophenylhydrazine method while the aromatic hydroxyl groups were determined with the Folin-Denis method. The anthrone test was used to determine carbohydrates, proteins were measured with the ninhydrin method and the Kjeldahl method, while fats and oils were measured as hexane extractables.

Evaluation of the hydroxyl amine test showed that the spectra obtained with the different molecular weight fractions in leachate corresponded to that of carboxyl groups attached to an aliphatic carbon chain , and was dissimilar from a carboxyl group attached to dihydroxyl benzene rings or hydroxylnapthalenes. It was further noted that the molar absorbance of most free volatile fatty acids is similar; however, this does not hold after introduction of a methyl or hydroxyl group in the aliphate carbon chain. Evaluation of the 2,4-dinitrophenyl hydrazine method showed that most carbonyl groups were present as aliphatic aldehydes and not as aliphatic ketones or aromatic aldehydes, a conclusion based on the specificity of the spectra of each group of compounds. The Folin-Denis test used to determine aromatic hydroxyl groups was found to be one of the most sensitive ones; however, the model compound has to represent the actual compound as closely as possible. The anthrone test for carbohydrate analysis was sometimes interfered with by the formation of brown colored products possibly as a result of the formation of furfural rings. Selection of a short hydrolysis time was found to eliminate this; aromatic hydroxyl compounds did not interfere with the test. The Lowry method, commonly used to measure proteins, was found to be greatly interfered by aromatic hydroxyl compounds; spiking of the sample with 1 mg/l tannic acid resulted in an apparent protein concentration of 3.8 mg/l and the less sensitive but more accurate ninhydrin test was therefore selected. The peptide bonds in this test are hydrolyzed by refluxing with 6 N HCl for 20 hours followed by addition of the ninhydrin reagent to the solution. Comparison of the Kjeldahl method and the ninhydrine method showed the results obtained with each test agree well with each other. Evaluation of extraction techniques using hexane and butanol followed by gravimetric determination of the residue showed that significant amounts of free volatile fatty acids are coextracted to result in erroneous high readings of the dried residue. The butanol, often used to extract fulvic acid, was found to preferentially extract aromatic hydroxyl compounds.

THE DETERMINATION OF CARBOXYL GROUPS WITH THE HYDROXYL AMINE TEST

THE HYDROXYLAMINE TEST

The hydroxylamine method used to determine carboxylic acids or carboxyl groups was first reported by Lossen in 1869 (Goddu *et al.*, 1955). However, it was not until 1934 that Feigl *et al.* (1972) used the hydroxamic acid together with complexed ferric iron as a spot test for organic acids. Keenan (1945) applied the method for determining acetic acid esters while Lipmann and Tuttle (1945) used it to determine the concentration of organo-phosphate esters at a wavelength of 540 nm. Sulfate and phosphate were found to interfere with the test by forming iron complexes competing with the hydroxamine acid complex for iron. Hill (1946) used the test to determine fats in ether solution using wavelength of 520 nm. Hestrin (1949) confirmed the findings of Lipmann and Tuttle (1945) that phosphate could reduce the color at 520 nm which interference could only be eliminated by using excess ferric chloride. Hestrin (1946) also noted that the pH of the final complex did not have any effect on the color between pH of 1.0 and 1.4 and that equal molar quantities of acetic, propionic and butyric acid resulted in the same amount of color. Branched acids however, gave a lower color intensity. Peptides did not interfere with the test, but ketonic substances did so slightly as a result of oxime formation, a result confirmed by Buckles and Thelen (1950). Goddu *et al.* (1955) conducted the test in anhydrous ethanol and found that at least a fivefold excess of iron is necessary for satisfactory final color development. Aromatic esters were found to have maximums at 530 nm while for aromatic esters it shifted to 550 nm.

Aksnes (1957) noted that excess hydroxylamine could cause a fading of the color by reducing the ferric to ferrous iron. Absorption readings therefore have to be made directly after the ferric chloride addition. The maximum color was formed at a pH of 1.4 and increasing the pH caused the absorption maximum to shift to a wavelength of 440 nm corresponding to a brown color instead of the purple color. Bayer and Reuther (1956) reported that acetic, propionic, butyric, valeric and caproic acid gave the same molar absorption. Lower molar readings however, were obtained with isobutyric and isovaleric acid and also with formic acid, confirming the results of Hestin (1949). Morgan and Kingsbury (1959) reported that all fatty acid esters gave a maximum absorption at 515 nm and that the chainlength has neither an effect on the wavelength at which the maximum absorption occurs nor does it affect the molarabsorption thereby confirming Bayer's results.

Goldberg and Spoerri (1958) noted that the presence of an electron releasing group in the molecule increased the rate of hydroxylamine formation which in turn corresponded to a lower color intensity. The closer the electron donor group is located near the carboxyl group, the more pronounced its effect, since methyl ester of toluate with the methyl group of the toluate in the ortha, meta and para position respectively, gave readings of 1.05, 1.29, and 1.42 times higher than found for methyl acetate. A similar result was obtained with aliphatic esters, thus explaining the lower reading obtained with branched acids. Aromatic compounds were found to give a hyperchromic effect as compared to aliphatic compounds. An electron pulling group, such as the hydroxyl group in methyl-p-hydroxybenzoate, resulted in an absorbance 1.79 times that of methylacetate, while a hydroxyl group in the para position gave an absorbance 1.45 times higher. An ortho methoxy group was less effective as an electron pulling group than a hydroxyl group and resulted in a reading 1.60 times higher than methylacetate. The authors did not investigate benzene rings with more than one hydroxyl group, nor did they investigate the different spectra since all compounds were measured at 540 nm.

Lehmann and Wilhelm (1967) found, contrary to Hestin (1949), that excess ferric iron concentrations did not influence the absorption. They did note a salt effect which caused a depression of the absorption. The effect was more pronounced with increasing salt content and increasing valence of the ions. Using ferric sulfate instead of ferric chloride also lowered the absorbance readings noticeably. The ferric chloride also resulted in the slowest fading of the formed complex as compared to ferric sulfate. The maximum color development with the ferric hydroxylamine acid complex occurred at a pH of 1.8.

Montgomery *et al.* (1962) proposed the hydroxylamine methods for measuring free volatile fatty acids in digester liquor and they included an esterification step with ethylene glycol in their method. More than 13 mg/l of acetic acid could be detected with this method; however, when colored samples were processed, a sample blank had to be included in order to obtain such sensitivity. The maximum color was formed at pH 1.6, and the absorbance was measured at 500 nm. They also noted, contrary to earlier findings, that the equimolar absorbances of propionic and butyric acid were only 88 and 87 percent of those obtained with acetic acid; the addition of the esterification step may well have caused this discrepancy with earlier investigations.

The above review showed that only one study used the hydroxylamine method for measuring fatty acids, while all other studies used the method to detect esters. Only one study investigated to some extent the response of aromatic esters; however, including the esterification step may well lead to different results. Other aromatic compounds that could be present in the fulvic and humic acids (Schnitzer and Kahn, 1972) were not tested.

PROCEDURES

The carboxyl test as used by Montgomery *et al.* (1962) to determine free volatile fatty acids was investigated and its applicability to aromatic acids was also evaluated. The reagents and procedures were as follows:

Reagents:

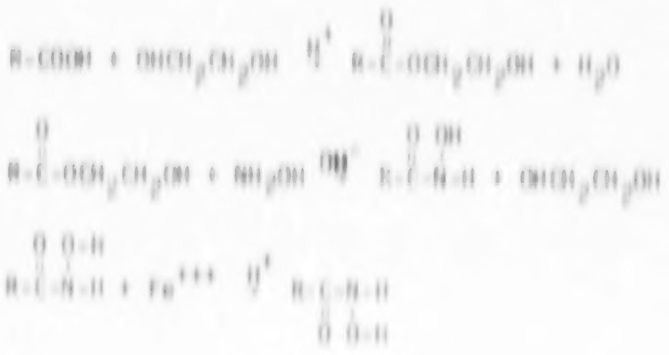
1. 50% sulfuric acid
2. ethylene glycol
3. 4.5 N NaOH
4. 10% hydroxylamine hydrochloride (10 gms in 100 ml HCl)
5. 20 gms ferric chloride, 20 ml concentrated sulfuric made up to 1 liter with distilled water

Sequence:

1. place 1.0 ml sample in tube
2. add 1.5 ml ethylene glycol

3. add 0.20 ml 50% H_2SO_4
4. stir mixture well for 2.0 min, at 25°C
5. cool to room temperature in water bath and add 0.50 ml hydroxylamine solution
6. add 2.0 ml NaOH solution, mix well and wait 1-2 minutes
7. add 10.0 ml ferric chloride solution
8. add 5.0 ml distilled water
9. read absorbance at 500 nm after 30 minutes

The reaction that takes place during the test is as follows:



Ester Formation: $R\text{-}COOH + OHCH_2CH_2OH \xrightarrow{H^+} R\text{-}\overset{\overset{O}{\|}}{C}\text{-}OCH_2CH_2OH + H_2O$

Hydroxamate Formation: $R\text{-}\overset{\overset{O}{\|}}{C}\text{-}OCH_2CH_2OH + NH_2OH \xrightarrow{OH^-} R\text{-}\overset{\overset{O}{\|}}{C}\text{-}\overset{\overset{OH}{|}}{N}\text{-}H + OHCH_2CH_2OH$

Color Formation: $R\text{-}\overset{\overset{O}{\|}}{C}\text{-}\overset{\overset{O-H}{|}}{N}\text{-}H + Fe^{+++} \xrightarrow{H^+} R\text{-}\underset{\underset{O}{\|}}{C}\text{-}\underset{\underset{O-H}{|}}{N}\text{-}H$

Colored 1/3 Fe^{+++}
Complex

COMPOUNDS GIVING A POSITIVE TEST

The results of the tests with the different free volatile fatty acids are shown in Figure 28. Acetic acid gave the highest molar absorbance while the other fatty acids such as propionic, valeric and caproic acid gave identical absorbances 79 percent lower than observed for acetic acid, which result agrees with Montgomery et al. (1962). Formic acid gave only 33 percent of the absorbance of acetic acid. As all absorbances were measured at 500 nm, the spectra of the different compounds were evaluated to determine whether 500 nm was indeed the optimum wavelength. The spectra of the different acids at a concentration of approximately 2.5 mM/l are shown in Figure 29a. The maximum absorbance is indeed observed between 500 and 510 nm which is indeed best suited for measuring the purple color of the solution. The introduction of a methyl or hydroxyl

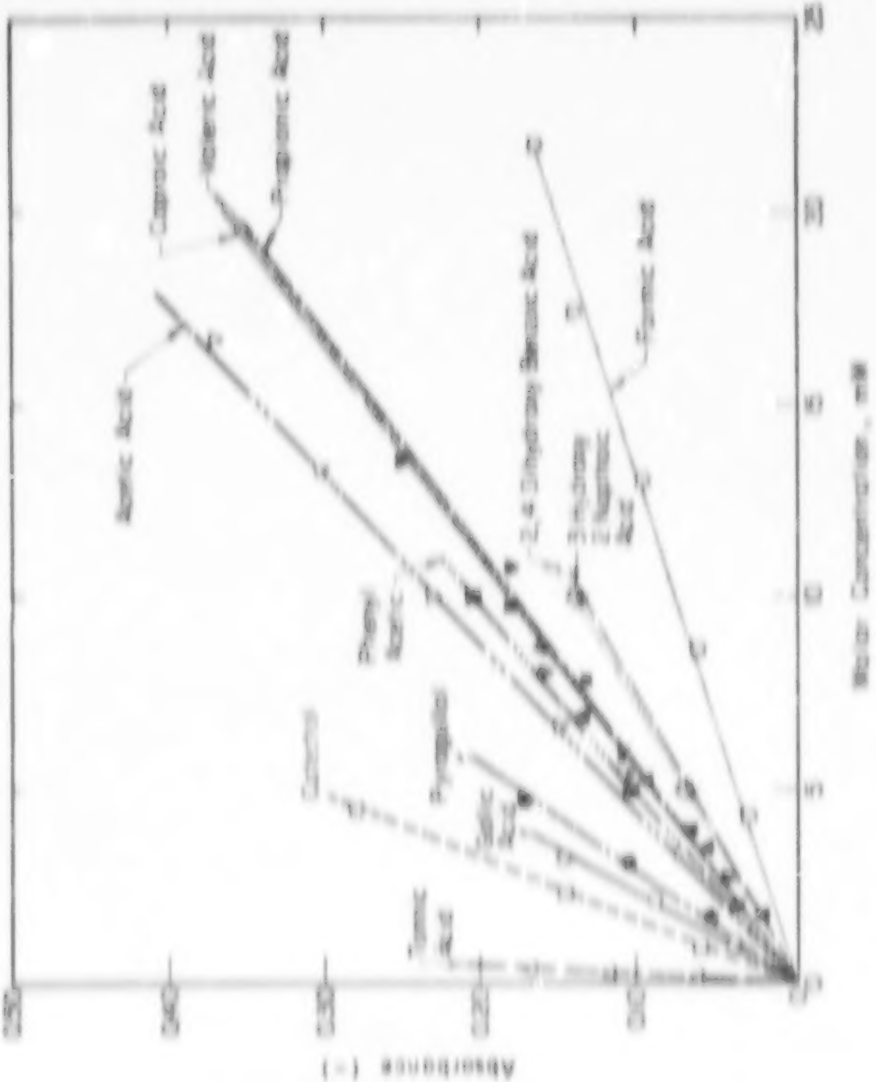


Figure 28. Molar Absorbances of Free Volatile Fatty Acids and Different Aromatic Compounds.

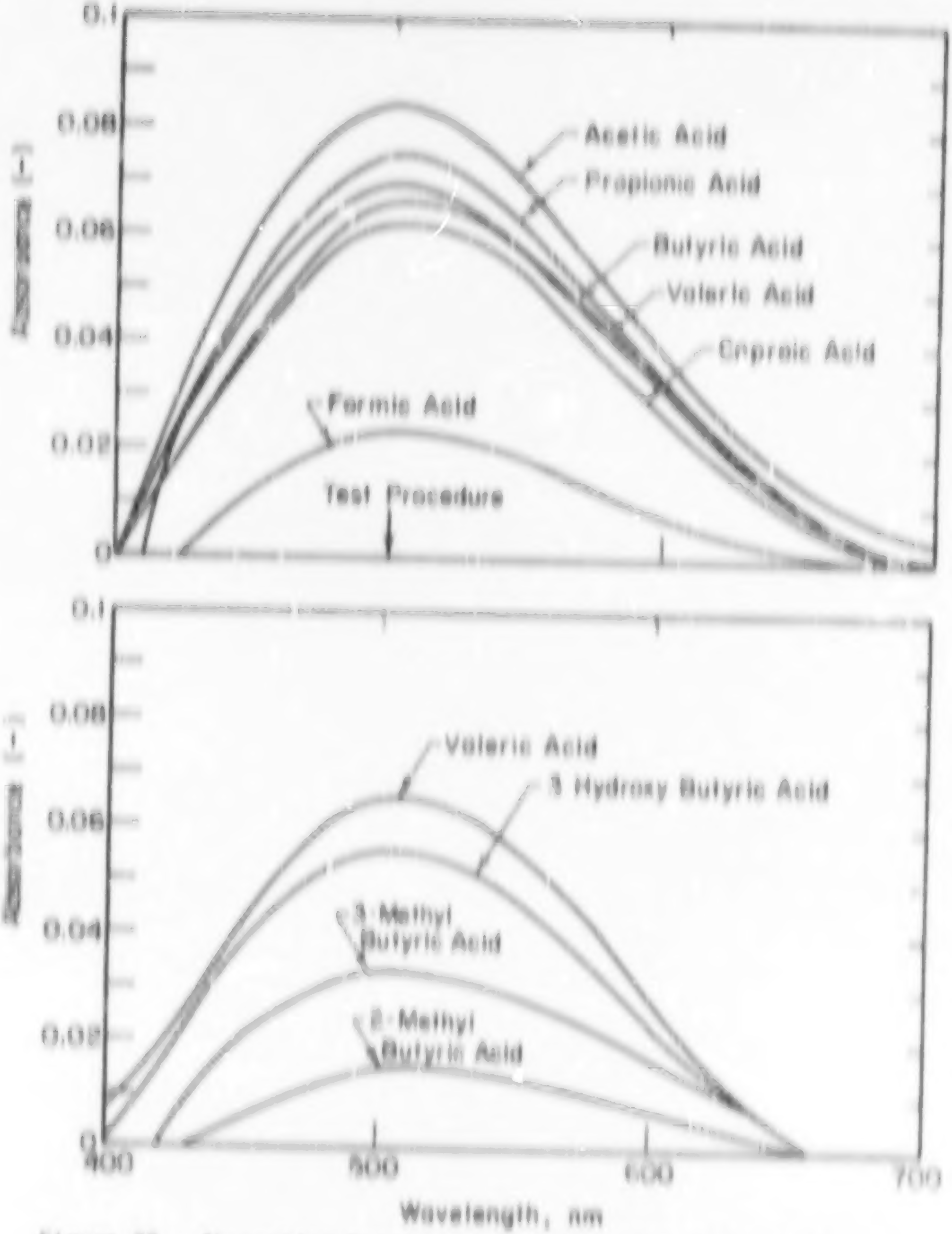
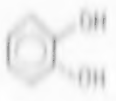


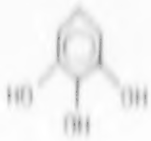
Figure 29. Absorption Spectra of Free Volatile Fatty Acids (A) and Branched Free Volatile Fatty Acids at a Concentration of Approximately 0.25 mmole/l

group into the aliphatic acid does not change the maximum absorption (Figure 29b) but only reduces its magnitude. Although the introduction of an electron donating group such as a hydroxyl group was expected to increase the absorption, such effect was not observed.

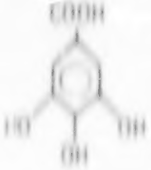
Several aromatic compounds which could be present in the fulvic and humic acid fractions were tested for their ability to give a color reaction:



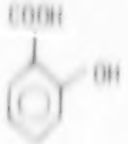
catechol



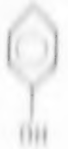
pyrogallol



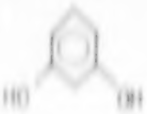
gallic acid



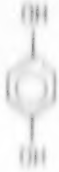
salicylic acid



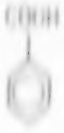
phenol



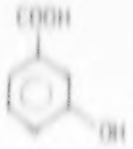
resorcinol



hydroquinone



benzoic acid



m-hydroxyl
benzoic acid

Only the first four gave a reaction (Figure 29) while no color development was observed with the latter five compounds. The color was different from that observed with the aliphatic acids since catechol gave a brown color, gallic acid and pyrogallol a green color, tannic acid a grey color, while the color from salicylic acid was violet and identical to those of the aliphatic acids. The spectra of the different aromatic compounds are shown in Figure 30. The spectrum of salicylic acid (800 mg/l) is almost identical to that of caproic acid (1000 mg/l) with the exception that the absorbance decreases less rapidly at higher wavelengths. The ratio of the wavelength at 560 to that of 500 nm is 88 for salicylic acid and only 74 for caproic acid. Such internal ratios can thus be used to

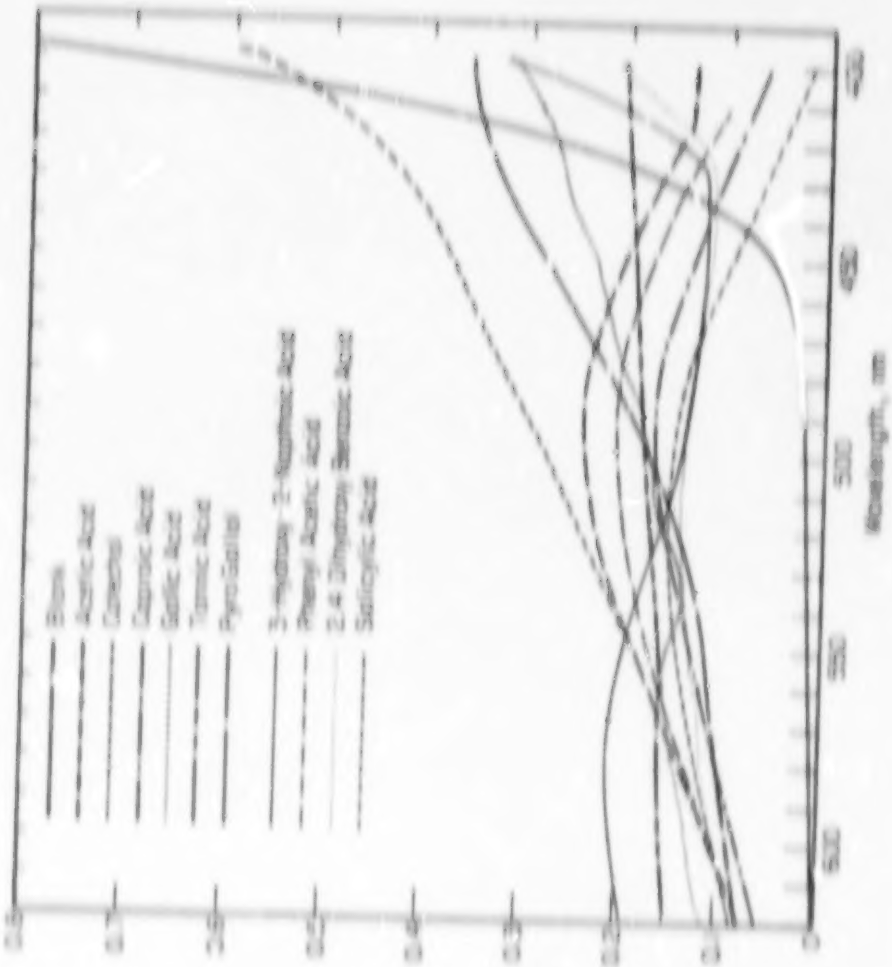
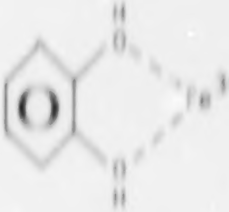
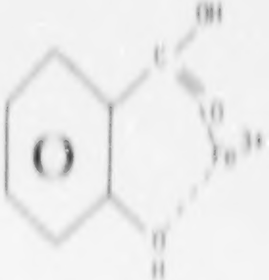


Figure 30. Absorption Spectra of Acetic Acid and Different Aromatic Acids and Hydroxyl Compounds

distinguish between different compounds. It should be realized however that the ratio can only be used when the absorption maxima are of equal magnitude. As catechol and pyrogallol are unable to form hydroxamates it was concluded that they formed a complex such as:



It is not yet known whether the color in salicylic acid is due to the reaction of the hydroxyl in the carboxyl group, which gives the normal hydroxamate colored complex, or a complex in which the Fe^{3+} is coordinated by the carbonyl of the carboxyl group and the oxygen of the ortho hydroxyl group.



To further evaluate this hypothesis a para nitro salicylic acid, or other ortho-h[illegible]yl benzoic acids in which the nitro group deactivates the COOH, will have to be tested. The inability of benzoic acid or m- or o-hydroxybenzoic acid to form a colored complex also has to be further investigated and it has to be determined whether this is due to the inability of the carboxyl group to esterify or due to the difficulty to form hydroxamic acids.

EFFECT OF VARYING FERRIC IRON CONCENTRATIONS

The effect of varying the concentration of the ferric iron solution was next evaluated. The Fe^{+++} concentrations chosen were 1.0, 2.0, 4.0 and 8.0 mM, while in the standard procedure the Fe^{+++} concentration is 3.7 mM. It was found that at the organic acid concentration studied, with a hydroxamate concentration of 0.50 mM in the final solution, Fe^{3+} ceased to be limiting beyond 8.0 mM Fe^{+++} (Figure 31). It was concluded that in the standard procedure with an Fe^{3+} concentration of 3.7 mM, small changes in Fe^{+++} concentration would have no noticeable effect on the color intensity.

FADING OF FORMED COLOR

Readings were taken at 0 and 30 minutes, 2 hours, and 16 hours with salicylic acid, formic acid and valeric acid in order to determine the stability of the different complexes. It was found that the color of salicylic acid reached its maximum at 30 min, then decreased about 25 percent after 16 hours (Figure 31). Both formic acid and valeric acid showed an increased color after 2 hours as compared to the reading after 30 minutes. After 16 hours the color of the valeric acid solution had not yet faded to the original 30 minute reading, while the color in the formic acid solution decreased to about 66 percent of its original value after 16 hours. It was therefore concluded that if readings are taken at 30 minutes ± 1 minutes, none of the colors have yet begun to fade and the least error is incurred with the different compounds. The results further show that not adhering strictly and systematically to this time limit will cause erroneous results.

EFFECT OF VARYING HYDROXYLAMINE CONCENTRATION

Several tests were made in order to determine the effect of changing the hydroxylamine concentration. Hydroxylamine concentrations of 5.0, 10.0, 20.0 and 40.0 mM were tested with a concentration of 400 mg/l of acetic acid. The normal procedure requires 35 mM NH_2OH, with concentrations referred to final volumes. It was found that at 5 mM the color formation

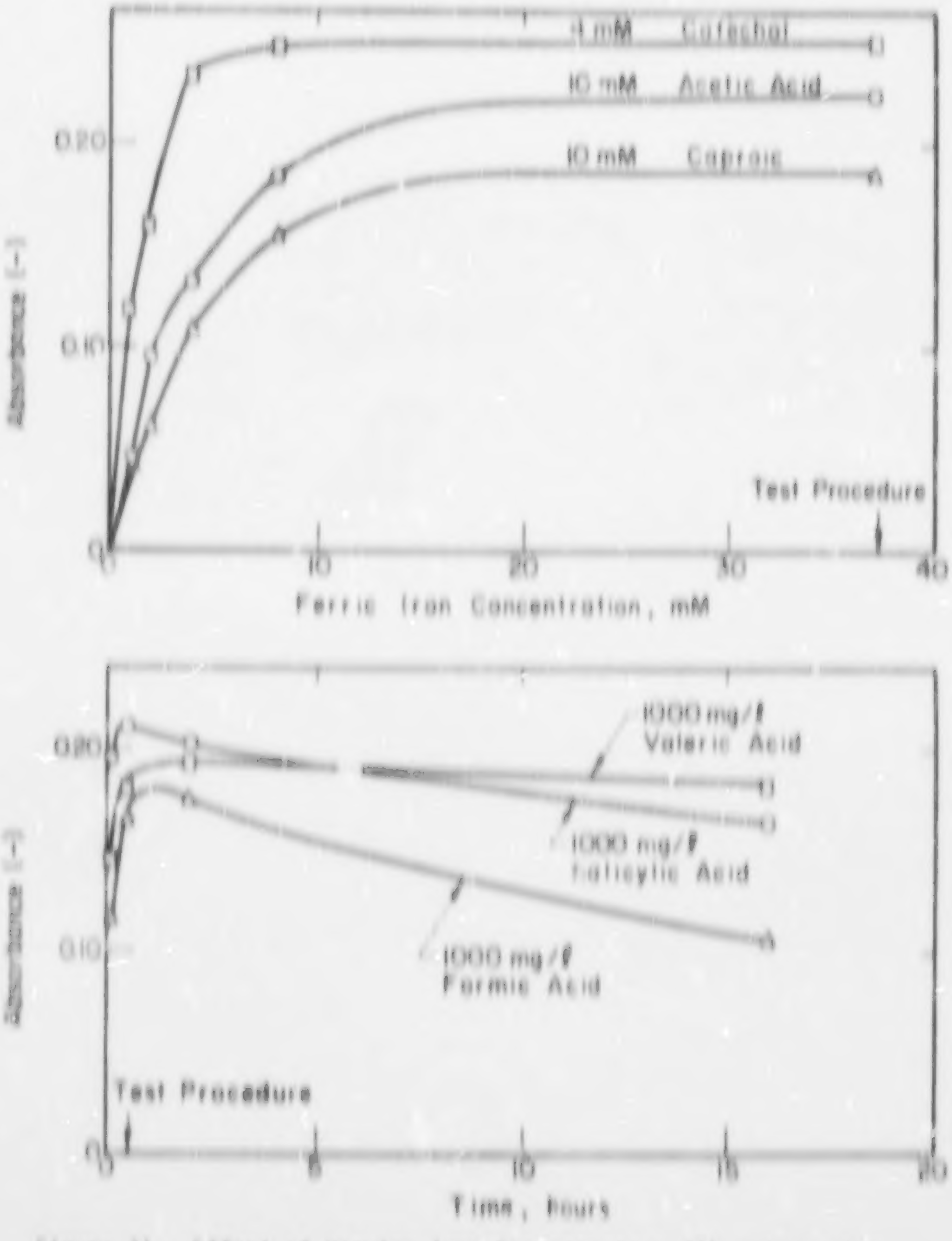


Figure 31. Effect of Varying Iron Concentration (A) and Varying Times Allowed for the Iron Complex Formation (B)

was limited by some factor other than hydroxylamine (Figure 32a), possibly the acetic acid concentration. It was therefore concluded that the selected hydroxylamine concentration would not cause any limitation in the hydroxamate formation.

EFFECT OF VARYING HYDROXAMATE FORMATION TIME

The length of the reaction period that the hydroxylamine is exposed to the carboxylester was also evaluated since increasing time periods would increase the hydroxamate formation. Tests were made at the standard temperature of 25°C for various time periods. The procedure was also tested at 100°C to determine if an increased temperature would affect the hydroxamate formation. It was found that at 25°C the reaction is essentially complete and stable after a few minutes and only a slight increase was observed over a following period of 15 minutes (Figure 32b). At 100°C, however, a decrease in the violet color was observed from 1.0 to 2.0 minute incubation, followed by a subsequent rise at 4.0 minutes due to the formation of a brown colored complex. It therefore appears that high temperatures promote the decomposition of the hydroxamate and hasten the formation of these brown colored products. Since it was shown that the reaction is nearly complete upon mixing at 25°C, the ferric chloride solution can be added after 1 to 2 minutes, thereby confirming the validity of the test procedures.

ANALYSIS OF LEACHATE SAMPLES

The leachate samples were tested and the amount of carboxyl groups were calculated as acetic acid equivalent. As the COOH measured in the membrane permeates is derived from fatty acids, a violet color was developed. The membrane retentates also gave a violet color and a spectrum similar to that of acetic acid, reflecting carboxyl groups which are probably associated with either aromatic carboxyl groups in combination with an ortho-phenol group, or from carboxyl groups attached to an aliphatic carbon chain which in turn may be attached to an aromatic ring. It is clearly not associated with dihydroxybenzoic acids or hydroxy napthoic acid.

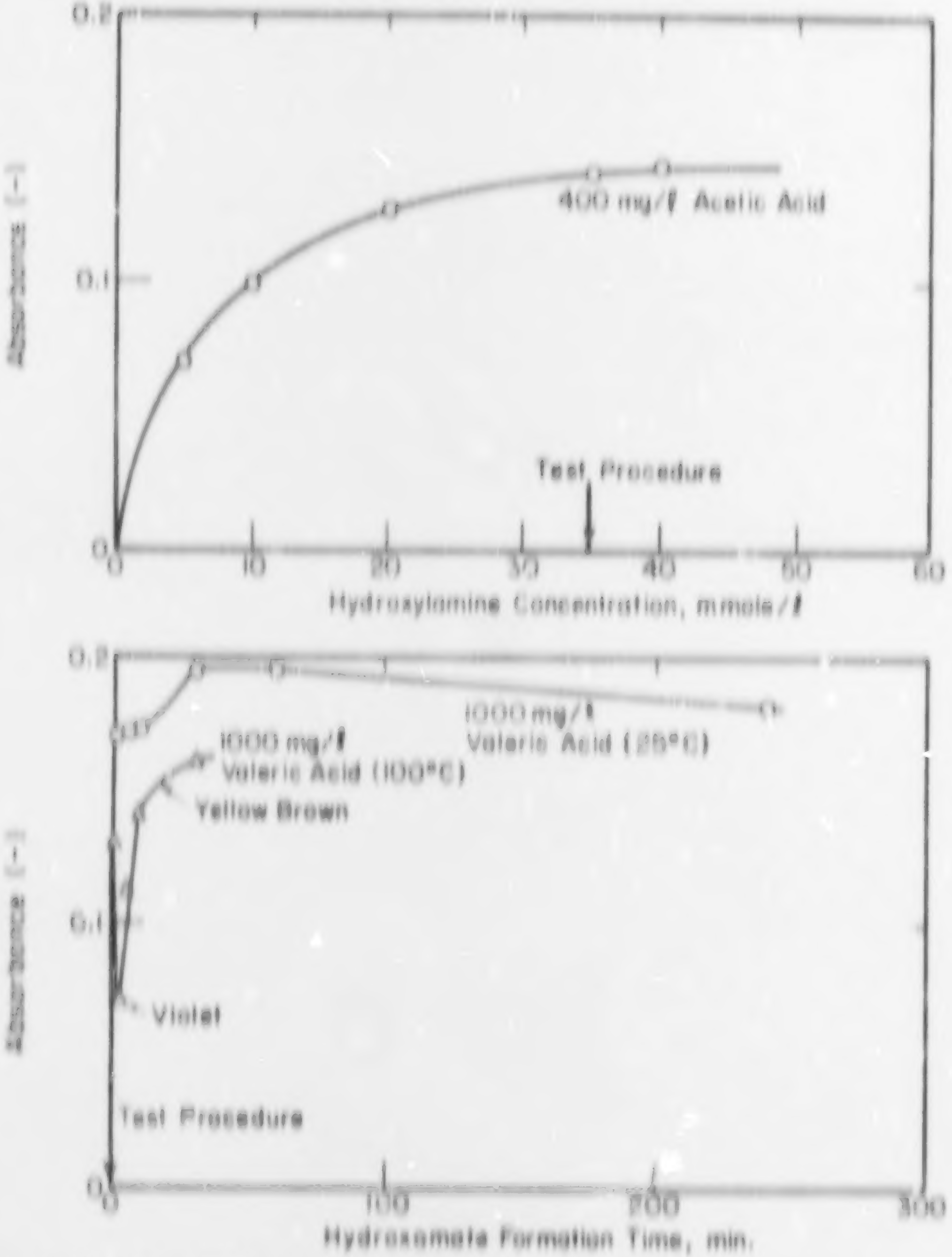


Figure 32. Effect of Varying Hydroxyl Amine Concentrations (A) and Varying Times Allowed for the Hydroxamate Formation (B)

CONCLUSIONS

The hydroxylamine colorimetric test for organic acids is reliable and reproducible for the compounds tested. It detects all aliphatic acids, with identical molar absorption coefficients except for acetic acid and formic acid. However,the molar absorbance decreases when methyl or hydroxyl groups are introduced in the aliphate carbon chain. This implies that the acid selected as standard has to resemble the most common acid present in the solution as closely as possible. The test also detects aromatic acids, especially aromatic carboxyl groups associated with an ortho phenol group, as commonly occurs in humic and fulvic acids.

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THE DETERMINATION OF FREE VOLATILE FATTY ACIDS BY GAS-LIQUID CHROMATOGRAPHY

THE GC ANALYSES

The use of gas-liquid chromatography has been growing rapidly because of sensitivity, resolution and speed of the analysis. It has thereby superseded the tedious and less accurate methods of column, paper and thin layer chromatography (Mueller et al., 1966). The first use of gas-liquid chromatography in the water pollution area was reported by Lamar and Goerlitz (1963), which method was followed by numerous other investigators. Due to advancing technology new liquid phases were developed that gave better separation of the mixture studied. Andrews et al. (1964) used a polyethylene glycol PEG 20,000 + H_3PO_4 on firebrick to determine acids in digesters while Hindin et al. (1964) used the lower polymer PEG 4000 + H_3PO_4 on Chromosorb W to determine the fatty acids in sewage. Murtaugh and Bunch (1965) measured the acids in effluent of several treatment plants by using a 6 mm by 1830 mm column packed with 20 percent (by weight) diethylene glycol adipate polyester and 2 percent phosphoric acid (85 percent concentrated H_3PO_4) on 60:80 mesh Chromosorb W with a helium gas flow rate of 60 ml/minute. The column temperature was maintained at 125°C for formic (C_1) to isobutyric (C_4) and 150°C for butyric (C_4) to caproic acid (C_6).

A similar glycol ester was used by Mateles and Chian (1969) to detect fatty acids in activated sludge by employing a 20 percent neopentylglycol succinate (NPGS) + 2 percent H_3PO_4 on 60:80 mesh firebrick adapted after Baumgardt (1964). A 3.17 mm x 1830 mm coiled stainless steel column (type 316) was installed. Compared with Baumgardt (1964) the oven temperature was 175°C instead of 165°C, injector temperature 205°C instead of 195°C, nitrogen gas carier flow of 39.8 ml/min instead of 26 ml/min and hydrogen flow rate to detector 18 ml/min instead of 20 ml/min. The air flow rate to the detector was 290 ml/min. In all cases sample sizes injected into the GC were between 0.5 and 1.0 microliter.

PROCEDURES

The columns used in the present study are similar to those of Mateles and Chian (1969). As solid support, acid washed Chromosorb P (AW) was employed. The hydrogen and air flow rates to the flame detector however were increased to 50 ml/min and 525 ml/min, respectively. Also, a molecular sieve trap was installed in the outlet of the nitrogen gas tank. This trap was regenerated for every other tank of N_2, i.e., 15.7 m^3 of N_2, in order to ensure a steady baseline in the recorder. The detector of the Hewlett Packard 5750B gas chromatograph was set at range 1 and an attenuation of 64 and 128. The Sargent-Welch SRG recorder was set at 1 mv. Phosphoric acid, that was added to the glycol stationary phase to improve the polarity, proved to be unstable at higher temperatures. Much better separation will therefore be obtained with a recently developed stationary phase FFAP (free fatty acid phase), a reaction produced between polyethelenglycol 20,000 and a nitroterephthalic acid. Improved separation was also obtained by using polyaromatic polymers as packing material. Van Huyssteen (1970) used 3 percent FFAP on a styrene-divinylbenzene copolymer and was able to analyze fatty acid concentrations in digesters as low as 5-10 mg/l. The order of magnitude of response per unit weight of acid was C_3, i-C_4, C_2, C_4, i-C_5, C_5, and C_6. Because the concentration of fatty acids in leachate is usually quite high the NPGS phase gave results accurate enough to not necessitate the more sensitive FFAP columns.

METHOD USED FOR CALCULATING FATTY ACID CONCENTRATIONS

Ideally, the area under a peak is proportional to the concentration of the compound. Because the area of a triangle is equal to the height times half the base width, the concentration is proportional to the height if the base width does not change appreciably between separate injection. This occurs if operating conditions are fixed, small samples are used and the gas flow rate is constant. The concentration of these acids in a sample can be calculated by comparing the peak height with peak heights of acids in a standard solution of a known concentration. Also, by adding increasing but known amounts of acids to the sample and by extrapolation to zero concentration one can calculate the concentration of the acids present. The most accurate method, which was also employed by Mateles and Chian (1969) is the use of an internal standard which has a larger retention timethan any of the known acids. After testing many substances, m-cresol was selected as the best internal standard at a concentration of 200 mg/l The calibration curves (Figure 33) relate the ratio of fatty acid/m-cresol peak height to the acid concentration in the standard. The calibration curves show that the i-C_4 acid can be detected most accurately to as low as 20 ppm. Hexanoic acid has the lowest sensitivity. Linearity of peak height with increasing acid concentration was observed in all cases. A similar amount of 250 mg/l m-cresol is added to the diluted sample to be analyzed and from the calculated ratio of acid to m-cresol peak height the actual fatty acid concentration is determined from the calibration curve.

OPERATIONAL COMPLICATIONS ENCOUNTERED

Most problems encountered with the gas chromatograph are related to 'ghosting' which is defined as the appearance of peaks with the same retention time as the previous injected acids when only water is injected. Baumgardt (1964) suggested that injector assembly and glass wool column plugs should be cleaned frequently. Distilled water samples should be injected to clean the column. Mateles and Chian (1969) minimized ghosting by coating the stainless steel wool in the injection port with 15 percent phosphoric acid. Ghosting is more pronounced in sensitive columns. Van Huyssteen (1970) experimented with this by injecting 2 μl distilled water after 2 μl of 25 mg/l C_2-C_6 solution or 1 μl of distilled water after 1 μl of 750 mg/l C_2-C_6 solution. Although Mateles and Chian (1969) usually worked in the range of 100-1000 ppm volatile fatty acids and a m-cresol concentration of 1000 ppm, our experiences with these high concentrations were less satisfactory. For example, at a concentration of 1000 ppm C_2-C_6 the ratio of acetic acid m-cresol in sequential injections could increase from 1.22 to 1.30 to 1.38, respectively. Other nonlinearities and ghosting phenomena were also experienced which led us to use lower concentrations of acids, in the range of 50 to 300 ppm and using the internal standard of 250 ppm m-cresol.

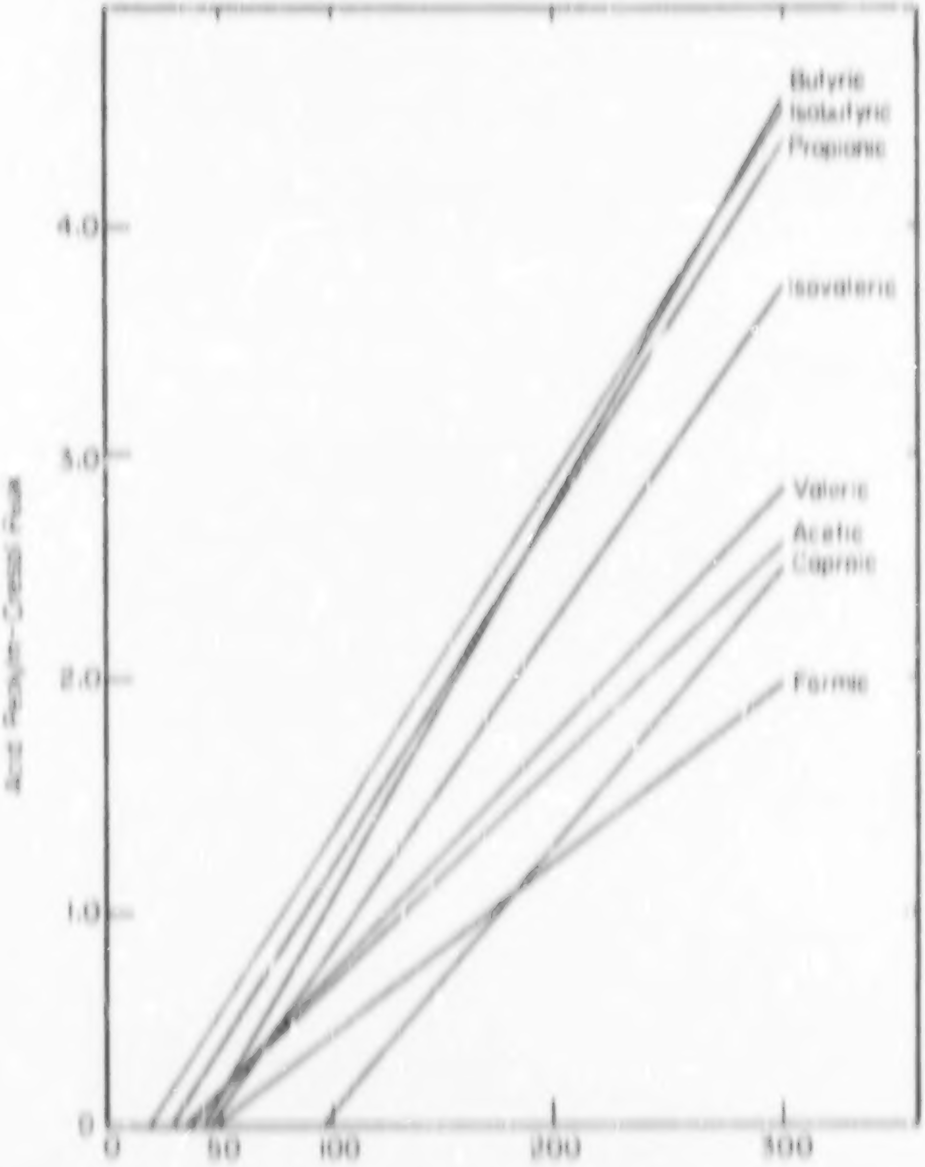

Figure 33. Calibration Curves for C_2-C_6 Volatile Fatty Acids

An example of the analysis of a leachate sample from the Boone County Field Site of SHWRL, EPA collected on October 2, 1972 is shown in Figure 34. The dilution employed is used to analyze for propanoic-, isobutyric-, isovaleric-, valeric-, and hexanoic acid. A dilution 4 times higher enables the determination of acetic- and butyric acid.

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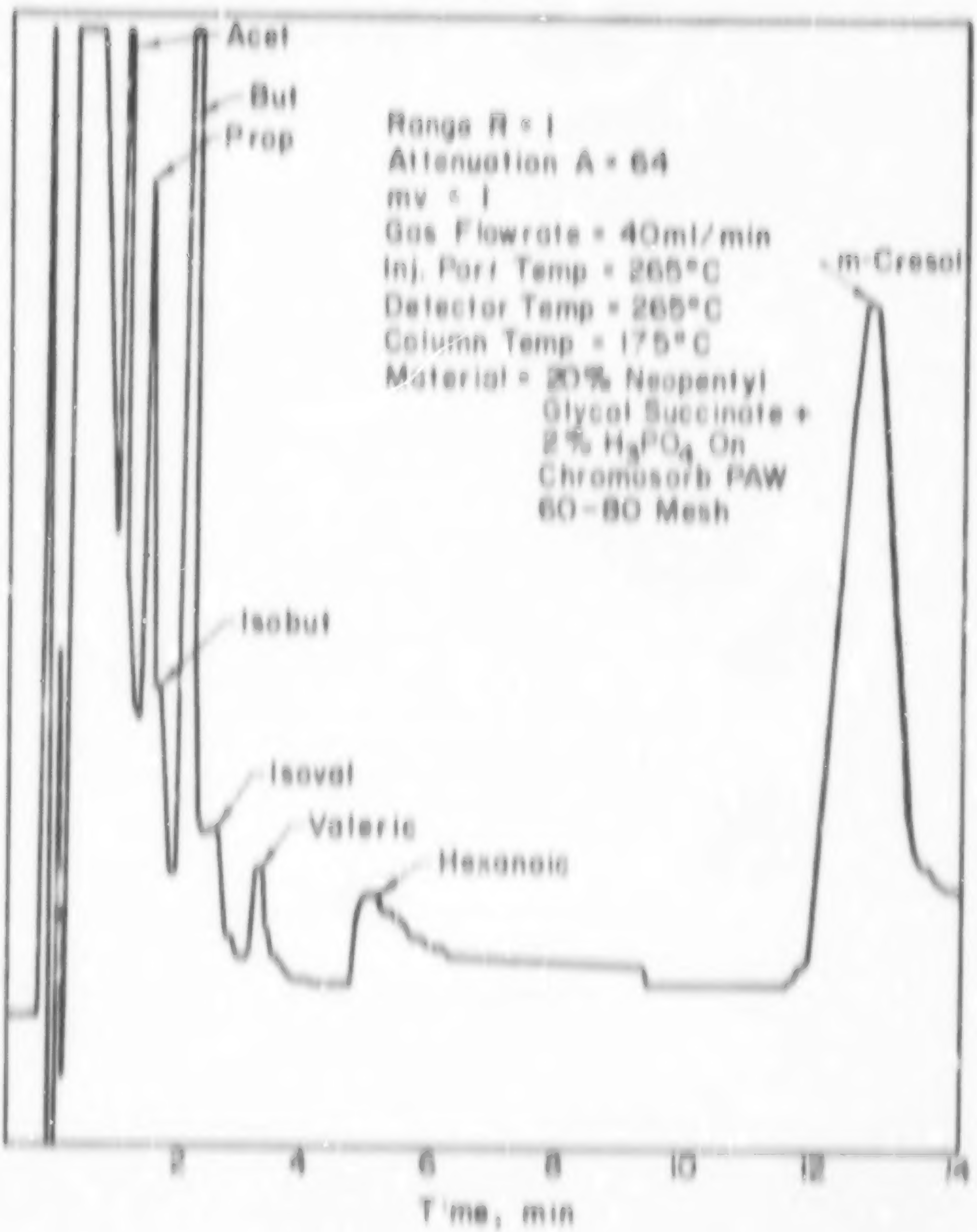


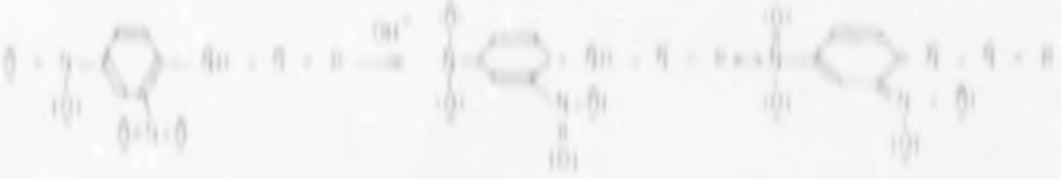
Figure 34. Gas Chromatogram of Free Volatile Fatty Acids Detected in Leachate of the Boon County-EPA Landfill

THE DETERMINATION OF CARBONYL GROUPS WITH THE DINITROPHENYL HYDRAZINE METHOD

THE HYDRAZONE METHOD

The most widely used methods for determining carbonyl groups are the oxime- and phenylhydrazone methods. The phenylhydrazone method which uses 2,4 dinitrophenylhydrazine as the active reagent to attach to the carbonyl group is interfered by excess reagent. This interference can be minimized by either selective extraction of the intense yellow derivative using a hydrocarbon solvent (Lohman, 1958) and measuring the absorbance at 340 nm or by addition of alkali to the solution of the derivative and measuring the wine red complex at 430-480 nm (Hanna, 1966). The molar absorptivity and the absorption maximum is dependent on the nature of the compound and is mainly influenced by the saturated or unsaturated character of the compound. The 2,4 dinitrophenylhydrazine method can also be used to precipitate and separate the different aldehydes that react with it using thin layer chromatography (Meyboom, 1968) or gas chromatography (Soukup, 1964; Fiacchia *et al.*, 1967).

The determination of small amounts of carbonyl compounds was first reported by Mathewson (1920) who measured acetone in water samples after extraction of the complex with carbon tetrachloride and measuring the absorbance at 435 nm. Barrenscheen and Dreguss (1931) and Barta (1938) used the purple color developed in alkaline solutions to determine concentrations of pyruvaldehyde and furfual respectively. The 2,4 dinitrophenylhydrazine has a proton donating character as a result of the two nitrogroups and will release a proton after addition of base, which causes the formation of wine red quinoidal compounds:



Iddles and Jackson (1934) used the precipitate of 2,4 dinitrophenylhydrazine and different ketones and aldehydes as a quanititative method to determine the concentration of the compounds. A saturated 2,4 dinitrophenylhydrazine solution in 2 NHCl was added to the aqueous sample after which a precipitate formed within 1 hour. An increased reaction time or increased temperature decreased the yield of the precipitate due to hydrolysis. When small amounts of aldehydes are present in aqueous solution, addition of 2,4 dinitrophenylhydrazine will only cause a turbidity and no precipitate in the sample, which turbidity can be measured quantitatively with a nephelometer.

Lappin and Clark (1951) determined the 2,4 dinitrophenylhydrazine complex of carbonyl compounds at a wavelength of 480 nm in an alkaline methanol solution. Excess hydrazine reagent gave a light yellow color in alkaline solution, the absorbance of which was corrected for by means of a blank. They added 1 ml of saturated 2,4 dinitrophenylhydrazine to 1 ml of sample dissolved in methanol. The sample with the methanol was first acidified by adding 1 drop of concentrated HCl. The mixture was heated for 5 minutes at 100°C or 30 minutes at 500°C and after cooling 5 ml of a methanol KOH (10 g KOH, 20 ml H_2O diluted to 100 ml with methanol) was added to give a wine red color. They reported that the molar absorption and the maximum wavelength were independent of the structure of the aldehyde compound.

Mendelowitz and Riley (1953) found that most aliphatic ketones gave a maximum absorption at 430 nm instead of 480 nm as used by Lappin and Clark (1951). They also noted that a fading of the color at 430 nm would occur after formation and that the absorption maximum is influenced by the structure of the compound. After addition of KOH to raise the pH of the solution a potassium chloride precipitate was observed. Jones et al. (1956) also found that the absorption maximum is influenced by the structure of the compound. They found that in neutral solutions aliphatic ketone hydrazones had absorption maxima at 364-367 nm which shifted to 430-440 nm in basic solutions. A second peak appeared at

526-535 nm which did not decrease rapidly with time. Aliphatic aldehyde hydrazones had maxima at 344-358 nm in neutral solutions which shifted to 426-435 nm in basic solutions. A second peak appeared at 515-523 nm which faded more rapidly with time than observed for the ketonic compounds. Formaldehyde hydrazone exhibited the most rapid decrease with 50 percent loss in 15 minutes. Phenyl acetaldehyde hydrazone was the most stable but still lost 30 percent of its color after 60 minutes. Aromatic ketone hydrazones had a maximum at 386-399 nm in neutral solutions which shifted to 458-490 nm in basic solution; no peak was observed at 520 nm. Aromatic aldehyde hydrazones absorbed at 381-390 nm which maximum shifted to 460-486 nm in a basic solution. Similarly to the aromatic ketones, no second maximum was observed at 520 nm.

Addition of water to the hydrocarbon-alcohol solvent reduced the problem of the KCl precipitates after addition of the basic methanol (Jordan and Veatch, 1964). They noted similarly to Mendelowitz and Riley (1953) that after the addition of base the absorbance must be determined within the interval of 8-15 minutes, since the color will fade beyond that time. They dissolved the sample in 5 ml 1:7 hexane-alcohol solution after which 2 ml of a saturated 2,4 dinitrophenylhydrazine alcohol solution and 0.1 ml of concentrated HCl was added. After heating at 55°C for 30 minutes it was diluted to 25 ml with a basic alcohol solution (59 g KOH, 180 ml H_2O, diluted to 1 l with alcohol). They observed a lower maximum wavelength for aromatic ketones than mentioned by Jones et al. (1956) since acetophenone and benzophenone gave maximum absorption at 446 nm and 430 nm respectively. They confirmed the earlier findings that aldehyde hydrazones fade more rapidly than ketonic hydrazones.

Sanders and Schubert (1971) modified the Lappin and Clark (1951) method by carefully acidifying the mixture of the aqueous carbonyl compound and the 2,4 DNPH methanol with 1.0 ml of 0.01 N HCl to result in a final pH of 2.5-2.7. After heating at 80°C for 10 minutes the sample was cooled for 10 minutes after which 5 ml of methanol KOH was added. After 10 minutes the absorbance was measured at 515 nm. Sugars did not interfere significantly in the pH range specified but did interfer at low pH because the conversion of the cyclic hemiacetal to acyclic aldehyde is accelerated. Aldehydes gave a relative constant absorption when the hydrazone was formed between pH 2.5 and 2.6, but the ketonic hydrazones gave better absorbances at pH 2.5 instead of 2.7 due to the slower rate of formation at the higher pH. The optimum heating temperature was 80°C since at 100°C, as recommended by Lappin and Clark (1951), sugars caused a substantial interference. A 10 minute boiling time at 80°C is sufficient to form hydrozones with ketones and aldehydes while only a fraction of the sugars have yet reacted.

PROCEDURE

The test is based on the formation of a red colored substance on the addition of base to the 2,4 dinitrophenylhydrazone in an alcohol solution (Lappin and Clark, 1951), and can be used as a colorimetric method for detecting carbonyl compounds in a water solution.

A saturated solution of 2,4-DNP was made up in 1.0 N HCl.
It was found that the 2,4 dinitrophenylhydrazone precipitated from the water layer during the test and that no quantitative colorimetric results were obtained indicating that the presence of methanol is required to increase its solubility. It was also found that the methanol contained too many impurities to give reliable results. One liter of methanol was therefore distilled from a 2,4-DNP-HCl mixture, in which the 2,4-DNP was recrystallized twice. The procedure is now as follows:

a) Add 1 ml sample
b) Add 1 ml saturated 2,4-DNP in methanol
c) Add 1 drop concentrated HCl (pH 2.5 - 2.7)
d) Heat at 50°C for 30 minutes
e) Cool, add 5.0 ml methanolic KOH
f) Read at 480 nm after 15 minutes

INCUBATION TIME

The effect of the elevated temperature during hydrazone formation was first studied. A precipitate was indeed formed, however too much methanol was found to evaporate. A lower incubation temperature of 50°C instead of the 80°C as recommended by Sanders and Schubert (1971) was therefore selected. The evaluation of the length of the incubation period with 15 mg/l pentanone showed that the reaction is essentially complete after a few minutes (Figure 35a), and that the recommended 30 minute period can be reduced if necessary. These results with regard to the length of the incubation time confirm the findings of Sanders and Schubert (1971).

FADING OF COLOR

The fading of the color beyond the recommended waiting time of 15 minutes after the color formation was found to be linear with time and acetone showed a decrease of 16 percent of the original color after 120 minutes (Figure 35b). All the leachate samples showed fading of the color. Using different test compounds it was noted that the fading was more rapid for aldehydes than for ketones, which emphasizes the importance of obtaining spectrophotometric readings at a standardized time, i.e. 15 minutes.

ABSORBANCE OF DIFFERENT COMPOUNDS

The absorbances of the different compounds are shown in Figure 36. The results contradict the results of Lappin and Clark (1951) but confirm the findings of Jones et al. (1956) in that aldehydes generally give higher absorbances than ketones and that aromatic structures also enhance the absorbance. Although insertion of functional groups in the benzene ring was reported to increase the absorbances, such was not found for piperonal which has two oxygen groups in the meta- and paraposition connected with a methyl group. When a methyl group is attached to an aromatic aldehyde group such as in acetophenone, the absorbance decreases due to the presence of the electron donating character of the methyl group. Acids having a carbonyl oxygen in the carboxyl group such as valeric and salicylic acid

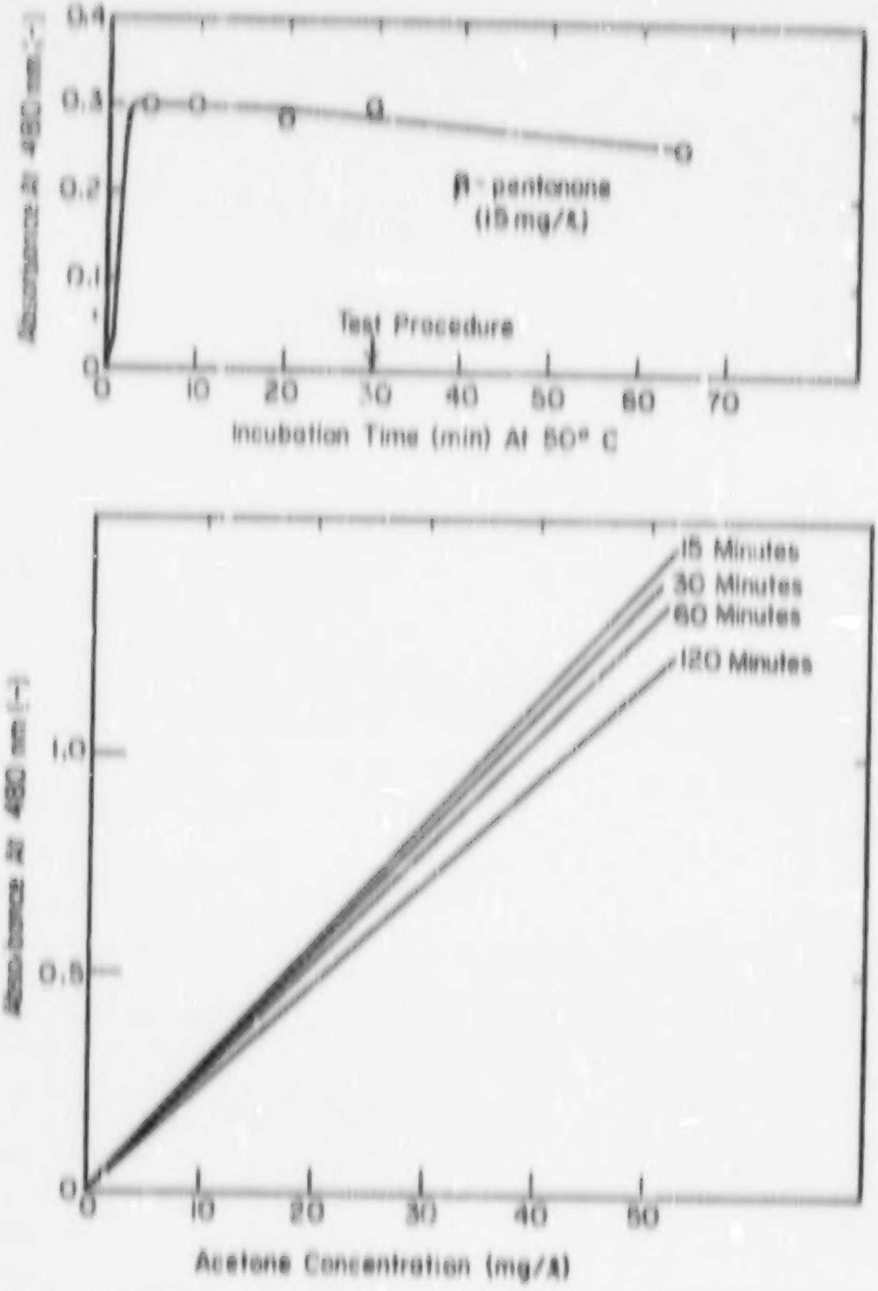


Figure 35. Effect of (a) Incubation Time on the Color Formation of Pentanone with 2,4 dinitrophenylhydrazine (b) effect of length of waiting time after the color formation on fading of color of acetone

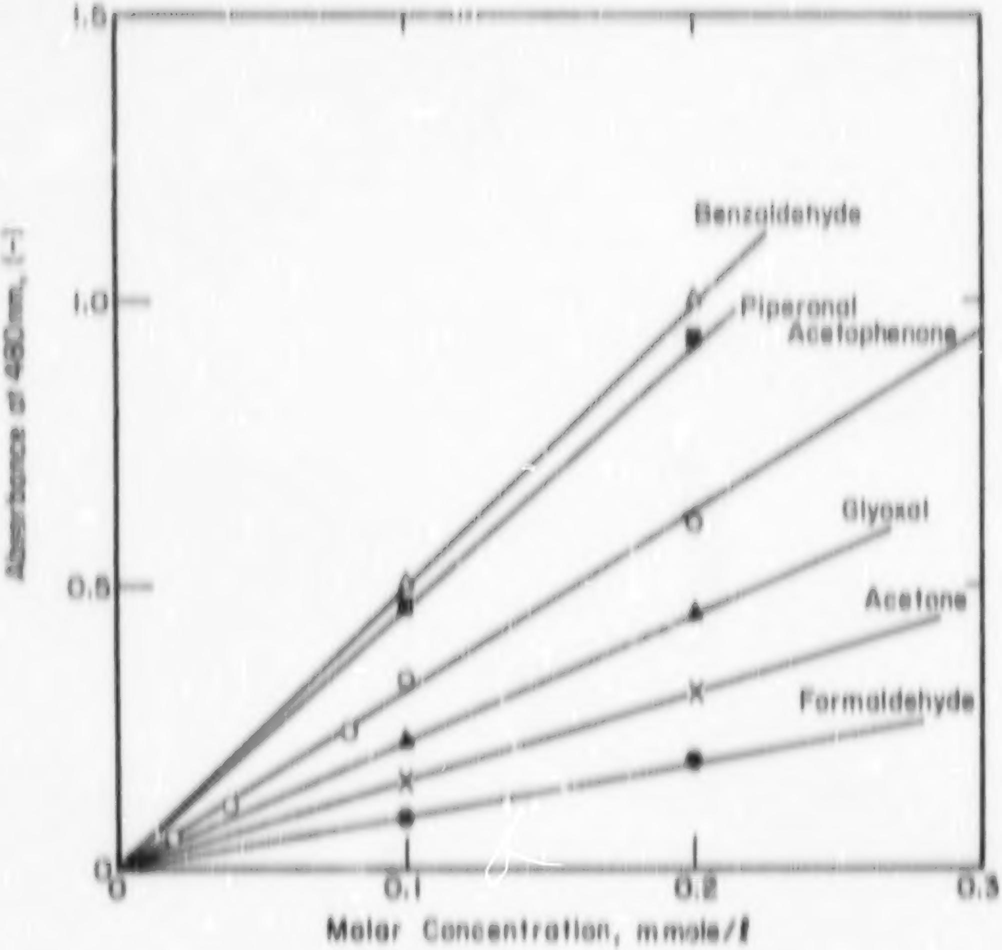


Figure 36. Molar Absorbances of Different Carboxyl Compounds

did not give any response in the test. Formic acid was the only acid that interferes but formic acid is present in the aldehyde form in only a small percentage. Dextrose at a concentration of 1000 mg/l did not give any response with the test. The spectraof the different compounds in alkaline media show that the wavelength of 480 nm as selected by Lappin and Clark (1951) is only valid for aromatic compounds (Figure 37) and p-dimethylaminobenzaldehyde did indeed have a maximum at 480 nm. In the absence of functional groups attached to the benzene ring the absorbance maximum even shifts to lower wavelengths, and maxima for benzaldehyde and acetophone were 464 and 458 nm respectively. In accordance with Jones _et al_. (1956) only a small shoulder was noted near 550 nm unless the aldehyde group attains a more aliphatic character such as with acetophone which has a noticable shoulder at 560 nm. Much higher wavelengths were observed for quinones which absorb at 540 nm and compounds with two aldehyde groups such as glyoxal which forms a cyclic hydrazone The aliphatic aldehyde compounds all have absorption maxima near 430 nm (Figure 38a) which maximum tends to shift to higher wavelength with increasing aliphatic chain length. In accordance with Jones (1956) it was noted that aliphatic ketones have a more pronounced shoulder at 540 nm than aliphatic aldehydes.

The spectra of the different molecular weight fractions in leachate are shown in Figure 38b and one can conclude that they resemble aliphatic aldehydes. The shoulder at 540 nm is less pronounced than found for propanal but is similar to that of valeraldehyde. The conclusion, that the carbonyl group is present as an aldehyde instead of a ketone, is important since oxidation converts the carbonyl into a carboxyl group which oxidation cannot take place with ketones.

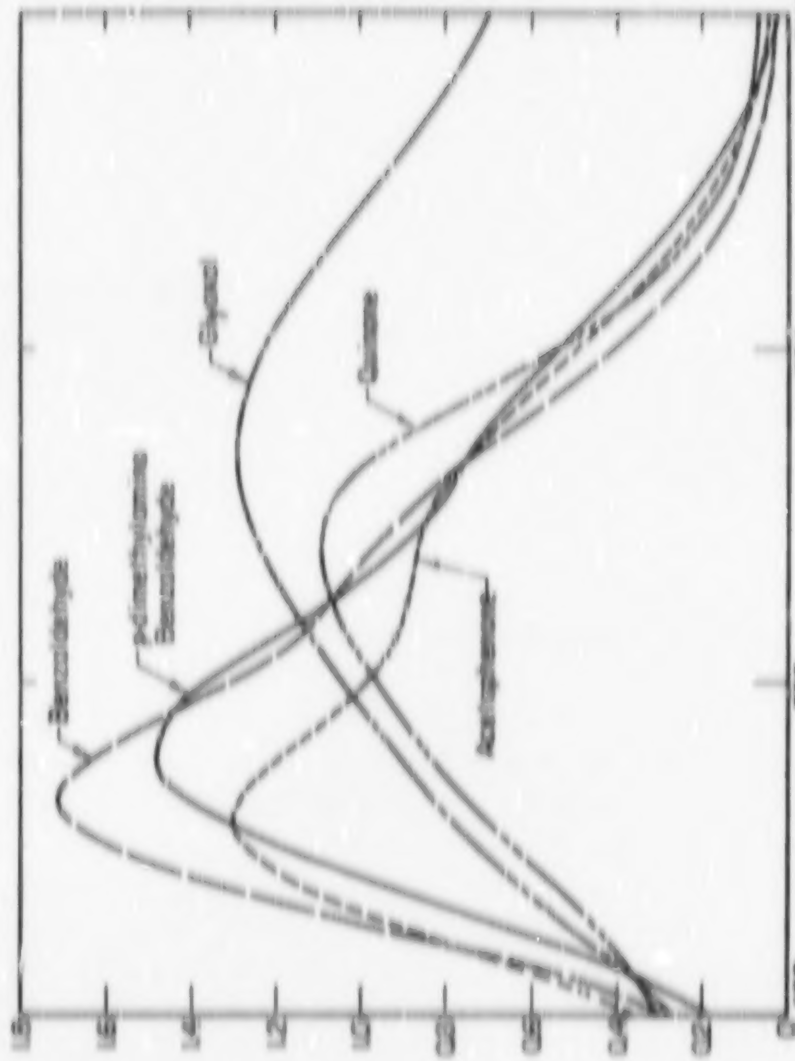


Figure 37. Absorption Specta of Different Aromatic Carbonyl Compounds

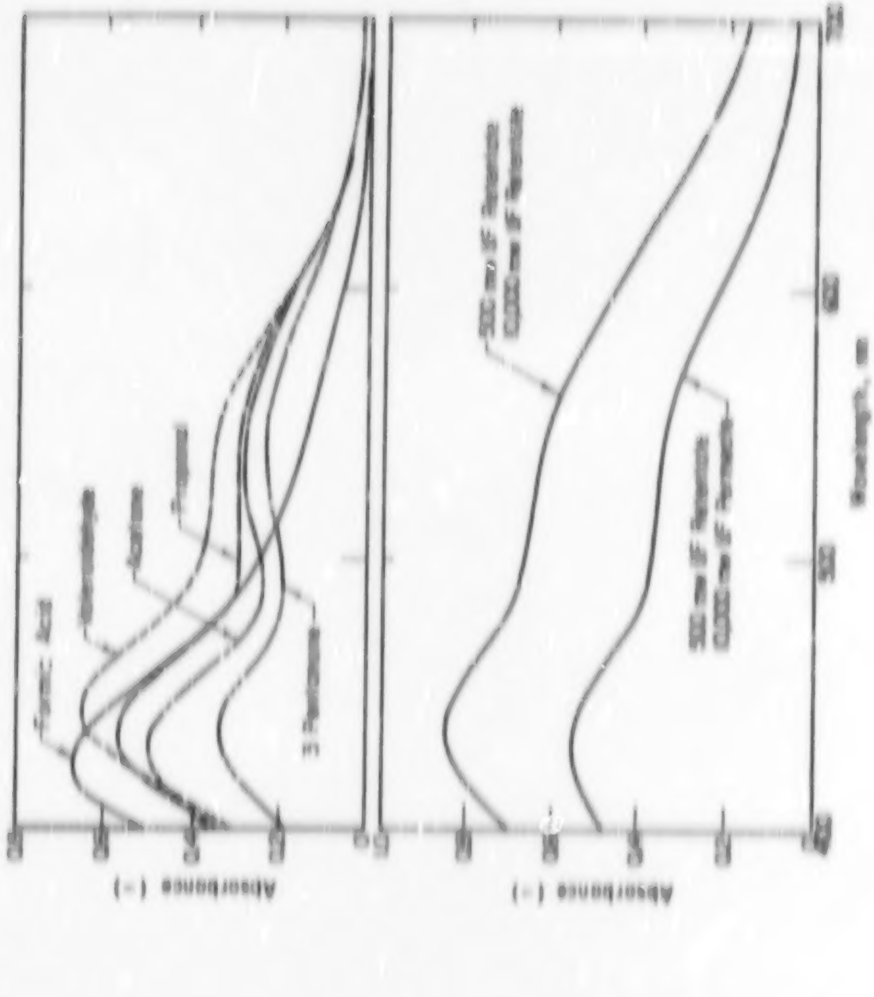


Figure 38. Absorption Spectra of Different Aliphatic Carbonyl Compounds and Leachate Fractions

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THE DETERMINATION OF AROMATIC HYDROXYL GROUPS WITH THE FOLIN-DENIS METHOD

THE FOLIN-DENIS TEST

The phenolic hydroxyl group concentration has been determined in humic acids by subtracting the value found for the carboxyl group (calcium acetate method) from the value found for the total acidity (bariumhydroxide method) which procedure is commonly used by soil scientists (Schnitzer and Khan, 1972).

When relatively small amounts are available, only colorimetric methods are applicable. The method used in this study was the Folin-Denis (1912) test which test is also listed in Standard Methods (1971) for the determination of lignin-tannin like material. The Standard Methods (1971) adapted modifications introduced by Berk and Schroeder (1942). The blue color is produced after addition of alkali to a mixture of the Folin-Denis reagent and the sample,which color is measured at 700 nm. The reaction mechanism is based on the oxidation of the phenolic hydroxyl group and the reduction of the phospho molybdotungstic acid. The method was tested by Smit et al. (1955) for different aromatic compounds having an hydroxyl group. He noted that more than one hydroxyl group in the benzene ring did not give a doubling of the color absorbance. He also concluded that the test is very accurate but that the standard should reflect the tested compound as closely as possible, since otherwise erroneous conclusions are drawn with regard to measured concentrations.

FOLIN CIOCALTEAU TEST

The Folin-Denis test was improved by Folin-Ciocalteau (1927) to eliminate a precipitate that sometimes formed during the test. The precipitate consisted of the sodium salt of the tungsten and molybdenum complex and it was found that the formation could be prevented by addition of the more soluble Li salts. The modified test also forms the basis for the Lowry protein test. The Folin-Ciocalteau reagent was used by Singleton and Rossi (1965) to measure phenolic compounds and by Kloster (1974) to measure tannins in water. The latter showed that reducing substances that are also present in the sample will increase the measured value. The largest interference is caused by ferrous iron and 2 mg/l will give a color equivalent of 1 mg/l tannic acid. The interferences are similar as those encountered in the phosphate test in which phosphate forms a complex with the molybdic acid which is then reduced with $SnCl_2$ or ascorbic acid to form a blue color. Interferences for the phosphate test include Fe^{2+}, sulfide and nitrite.

PROCEDURES

Since no precipitation was observed with the Folin-Denis reagent it was used instead of the more complicated Folin-Ciocalteau test. The procedure is identical to the tannin-lignin test as listed in Standard Methods. Since most of the organic fractions measured showed a slight leveling off at 620 nm (Figure 39), this wavelength was selected for all measurements.

RESULTS

To obtain a clearer understanding of the reaction, different aromatic compounds having more than one hydroxyl group in the benzene ring, were tested. As shown in Figure 40, the intensity of the colored product depends on whether the location of the OH groups is in the ortho, meta, or para position. When the ring has three OH groups, such as in gallic acid one of the groups may not participate in the reaction. Further tests are necessary to gain a better understanding of the mechanism of this test. It can be concluded that this is a sensitive test for phenolic hydroxyl compounds, however, the standard compound has to reflect the composition of the humic and fulvic acids as closely as possible.

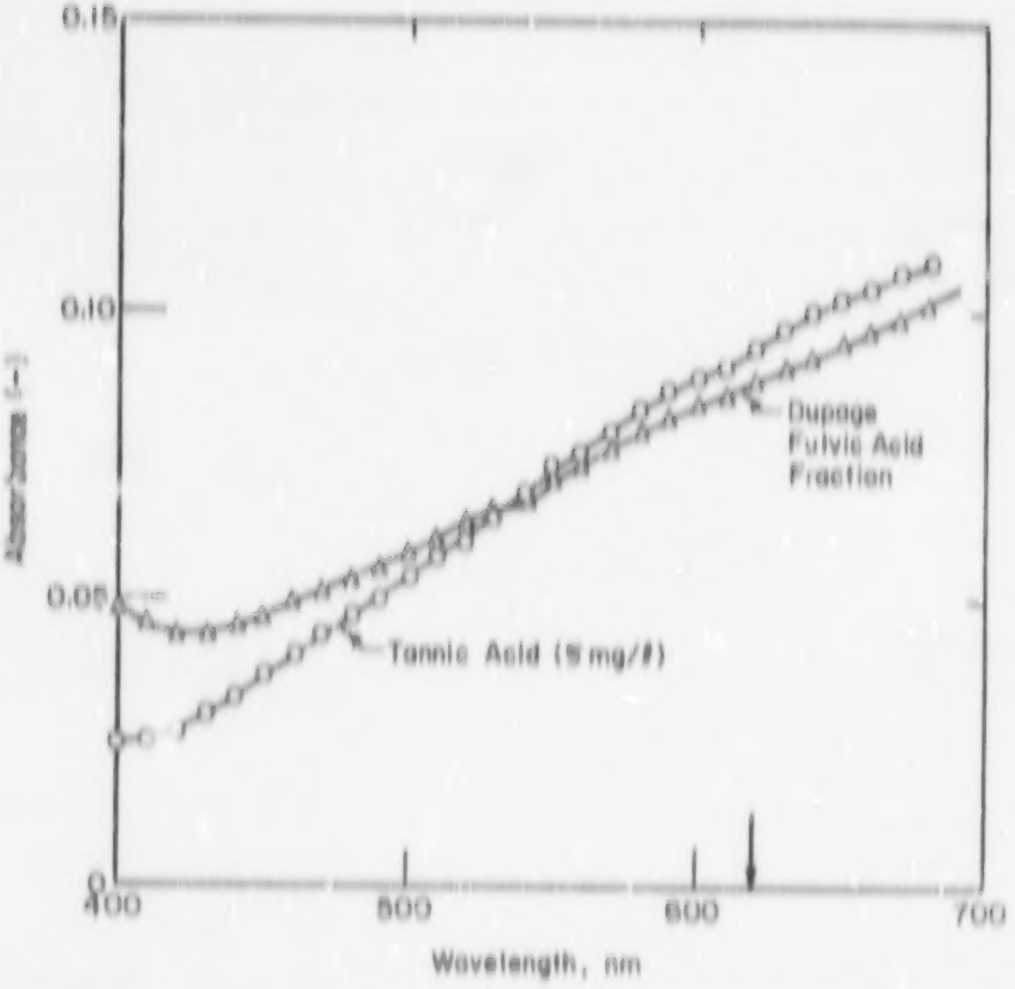

Figure 39. Spectrum of the Fulvic Acid Fraction of the Dupage Landfill Leachate and the Standard Tannic Acid

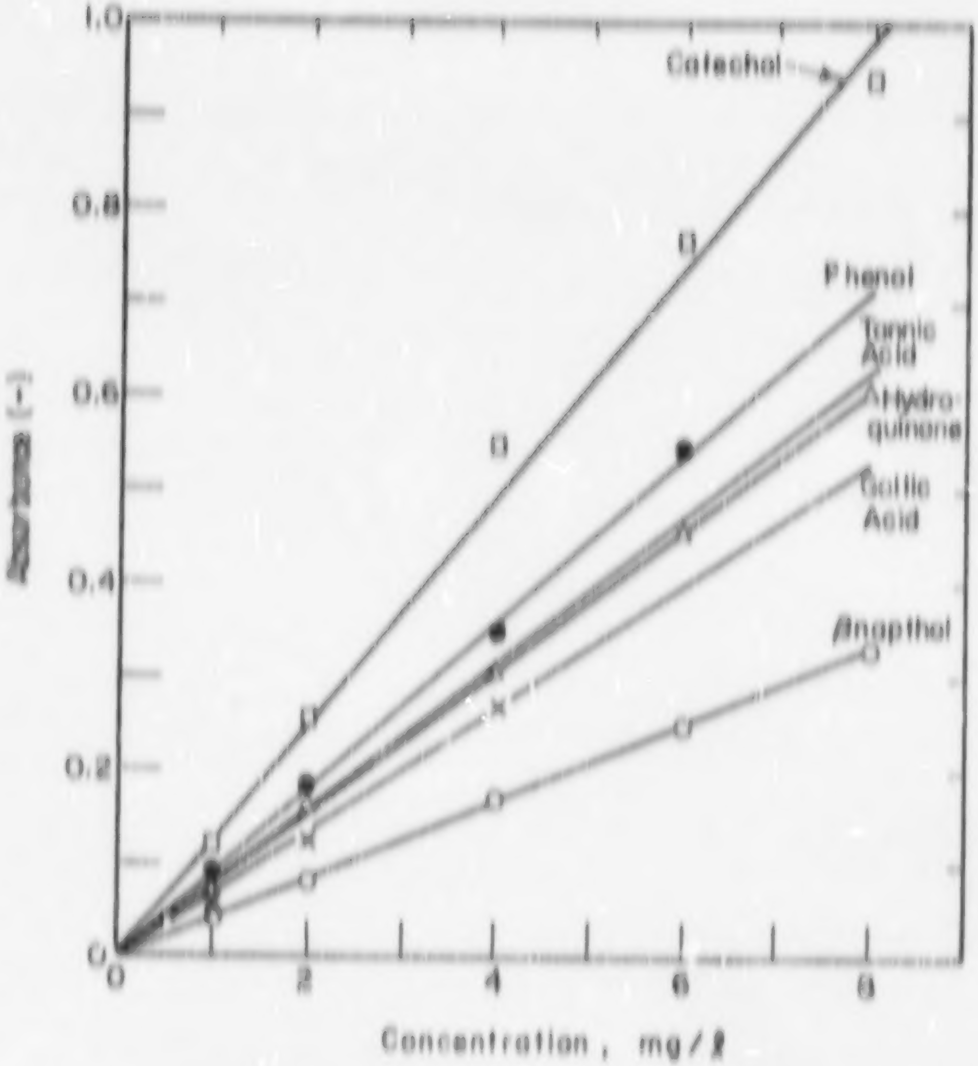

Figure 40. Absorbance of Different Aromatic Hydroxyl Compounds

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THE DETERMINATION OF CARBOHYDRATES WITH THE ANTHRONE TEST

THE ANTHRONE TEST

The most common colorimetric methods of determining carbohydrates are the phenol sulfonic acid method and the anthrone method. Because the former method procuces a yellow color the reaction might be interferred witn by the yellow brown color of the leachate; the anthrone test on the other hand gives a blue-green color reaction. The color in this test has been attributed to the reaction product of 5-hydroxymethyl furfural or furfural and anthrone (9,10-dihydroxyl-9-oxoanthracene) a reduction product of anthra quinone in an acid solution (Dreywood, 1946). Glucose can further decompose to laevulinic acid, black humic products, formic acid and formaldehyde (Amihoff, 1970). The different sugars display a wide range of color intensities, and in this respect the test is inferiur to the phenol-sulfuric acid test in which the different sugars give a more identical response. It is therefore of great importance to select a standard that closely resembles the building units of the polysaccharide to be determined. The test has been applied successfully in the determination of hexosen, pentosen, deoxy sugars, hexuloses, heptuloses, glycogen, and cellulose(Seifer et al, 1950; Black, 1951). Some compounds will interfere slightly; for example, fatty acids, some proteins (Peterson and Rose, 1951) and a high salt content (Lanner, 1960). No interferences were given by 1 M Na, 1 M K, 1 M PO$_4$, 1 M NH$_3$, 0.1 M Cl, 0.05 M Mn, 0.01 M Ca and 0.01 M Ba (Koehler, 1952). Precautions have to be taken when particulate matter is analyzed (Biggs and Wetzel, 1968). When cellulose or glycogen like material has to be determined, the solution should be boiled in acid for a relatively long period as compared to single sugars. Boiling during an extensive period, however, polymerizes the furfural units into brown humic complexes which will interfere with the analysis.

PROCEDURES

The tests reported here have been conducted according to the recommendations of the International Biological Program, (Golterman and Clymo, 1969), in which 5 ml of sample is boiled in 25 ml 58 percent sulfuric acid containing 140 mg/l anthrone reagent for 15 minutes. The anthrone reagent is obtained by dissolving 0.1 g of anthrone in 500 ml concentrated H_2SO_4 to which 200 ml H_2O and 1 g of thiourea is added as an oxidant. The boiling time of 15 minutes was selected because higher readings are obtained when heated longer than that period due to a brown color formation which absorbs at 540 nm instead of 620 nm.

RESULTS

When the test was run on the whole leachate and the different molecular weight fractions, all resulted in a blue color and the absorption spectra were similar to that of glucose, indicating that glucose is a good standard to use. The color was less well developed in the 500 MW permeate as compared to other membrane fractions as it had traces of a brown color corresponding to a shift in the absorption maximum from 620 nm to 540 nm, which change became stronger after 15 minutes boiling. All fractions showed a continuous increase in absorbance with increasing boiling time (Figure 41), probably due to the formation of brown humin products. The most rapid increase was observed for glucose when the boiling time was increased from 15 to 30 minutes. This resulted in lower apparent concentrations for each of the fractions when expressed as glucose equivalent at the 30 minute as compared to the 15 minute digestion time. The values after 4 hours boiling were higher than the 15 minute values when expressed as glucose equivalent since the absorbence of each of the fractions would increase while the values for glucose would remain constant. Since a 15 minute boiling time would result in a minimum change toward a brown color, this period was therefore selected as the optimum boiling period. Aromatic hydroxyl compounds that resemble the composition of the low molecular weight fulvic acid fraction did not cause significant interference in the test (Figure 42). In combination with glucose however, they may

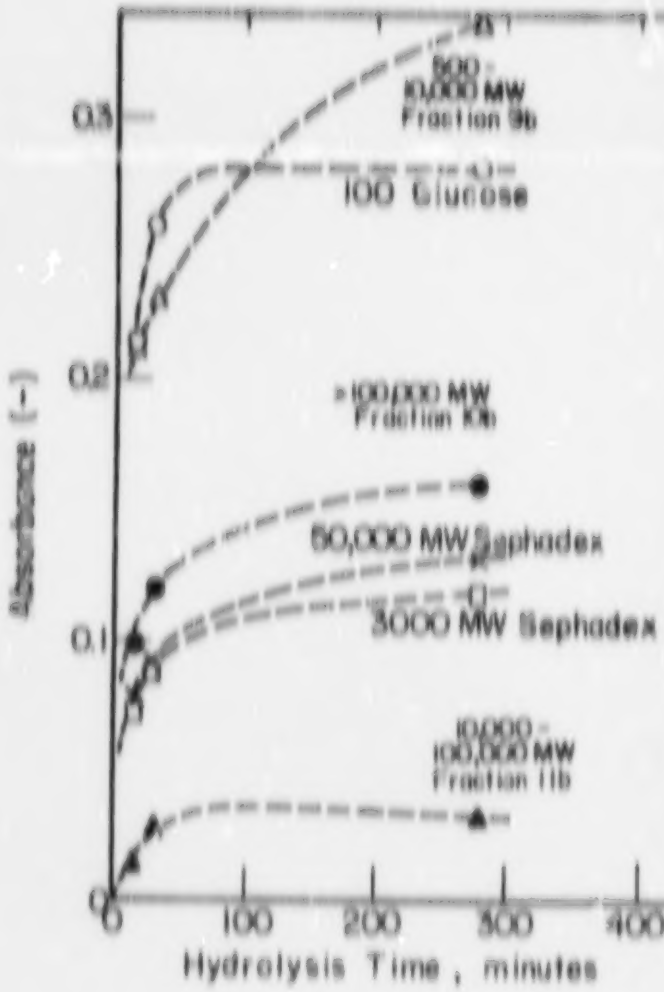

Figure 41. Effect of Hydrolysis Time on Absorbance of Different Molecular Weight Fractions in Leachate

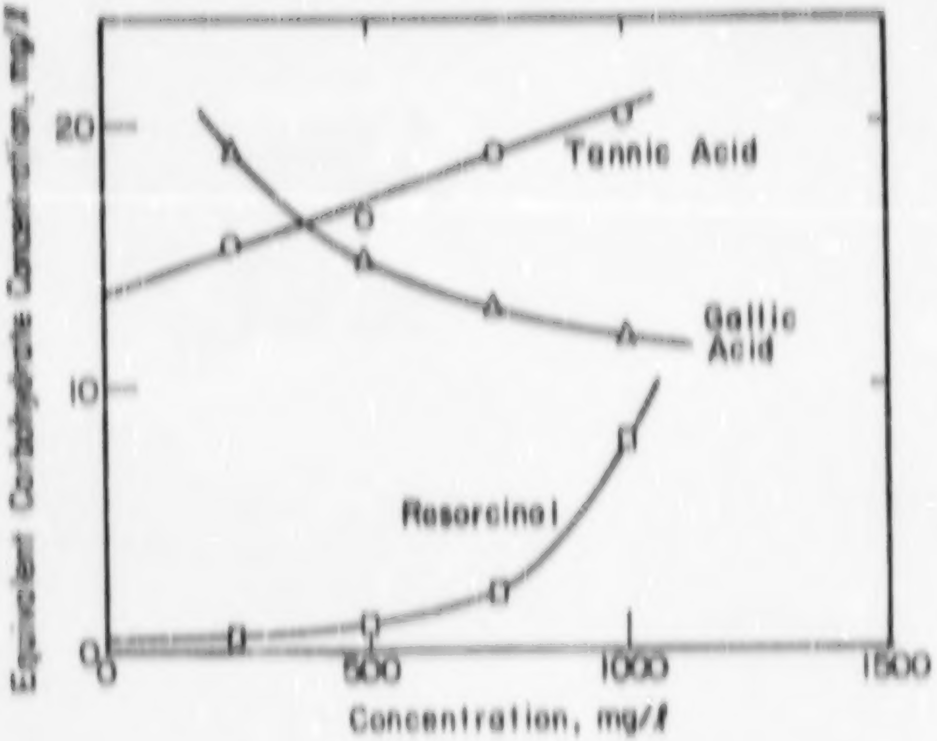

Figure 42. Interference in Carbohydrate Analysis by Aromatic Hydroxyl Compounds

cause formation of brown products (Feeny, 1969) and may have partially caused the brownish color development in the 500 MW UF permeate.

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THE DETERMINATION OF PROTEINS WITH THE LOWRY, NINHYDRIN AND KJELDAHL METHOD

THE LOWRY METHOD

The Lowry method is the most commonly employed protein test in laboratory determinations. The test was developed by Folin and Ciocalteau (1927) and significantly modified by Lowry et al. (1951) who added the copper reagent used in the biuret reaction. The test is based on the reduction of phosphotungstic and phosphomolybdic acid and the concurrent oxidation of the phenolic hydroxyl group of aromatic amino acids that usually make up 10 percent of any protein, resulting in a reaction product that gives a blue color. Miller (1959) modified the test by using an elevated temperature (50°C), a smaller volume of a more concentrated alkaline copper reagent, and a larger quantity of a more dilute phenol reagent. Schacterle and Pollack (1973) simplified the procedure even further by using only two reagents.

The amino acids mainly responsible for the reaction are tyrosine, tryptophan and the purines, guanine, xanthine and uric acid. A recent evaluation of the Folin test by Shao-Chia and Goldstein (1960) reported that although a large part of the color is caused by the reaction of the phenolic hydroxyl in the aromatic amino acids, any peptide bond will yield some color depending on the sequence of certain amino acids. They found that some amino acid sequences have a larger impact on the color than others. Hydrolysis reduced the color of albumin by more than two-thirds as it eliminated the protein bonds. Cleavage of the disulfide bonds in insulin by oxidative reactions also gave a loss of about one-third of the total color. Ji (1973) reviewed the interferences of the Lowry test and stated that some carbohydrates, lipids, di- and tri-carboxylic acids and single amino acids like glycine and glycylglycine interfere with the test. Other limitations of the test are that the amount of color varies with different proteins while other aromatic compounds present in the sample contribute to the color. The main advantages of the test are [1] that it is as sensitive as the Nessler reagent but does not require prior digestion of the sample, [2] it is several fold more sensitive than the ninhydrin method, [3] it is 10 to 20 times more sensitive than measurement of the UV adsorption and a hundred times more sensitive than the biuret method (Lowry *et al*., 1951; Young, 1963).

In the water pollution area the Folin test has been used extensively by Orme-Johnson and Woods (1964), Walters *et al*. (1968) and Rebhun and Manka (1971). The latter used it for determination of proteins in treatment plant effluent. Phenolic hydroxyl groups in humic acid in the effluent, however, will greatly interfere with the test. Because high protein values were initially obtained in this study, as compared to the protein concentrations calculated from the organic nitrogen values multiplied by 6.25, the Lowry test was evaluated with respect to interferences from aromatic hydroxyl compounds.

When increasing amounts of tannic acid, containing numerous aromatic hydroxyl groups, were added to a 1:100 and 1:200 diluted leachate sample, increasing apparent amounts of proteins were measured with the Lowry test (Figure 43a). It was found that 1 mg/l tannic acid gave a response identical to 3.8 mg/l proteins, clearly illustrating that this protein test is interfered with by the presence of aromatic hydroxyl groups. When the values obtained with the Lowry test using bovine albumin as a standard and the aromatic hydroxyl test using tannic acid as a standard (Figure 43b) a similar result was obtained as 1 mg/l tannic acid corresponded to 3.8 mg/l proteins, indicating that a relatively low protein content reactive to the Lowry test is present in leachate.

THE NINHYDRIN METHOD

A test which is very accurate but several times less sensitive than the Lowry test uses the reaction between ninhydrin (triketohydrindenehydrate) and the individual amino acids. Moore and Stein (1948) tested the

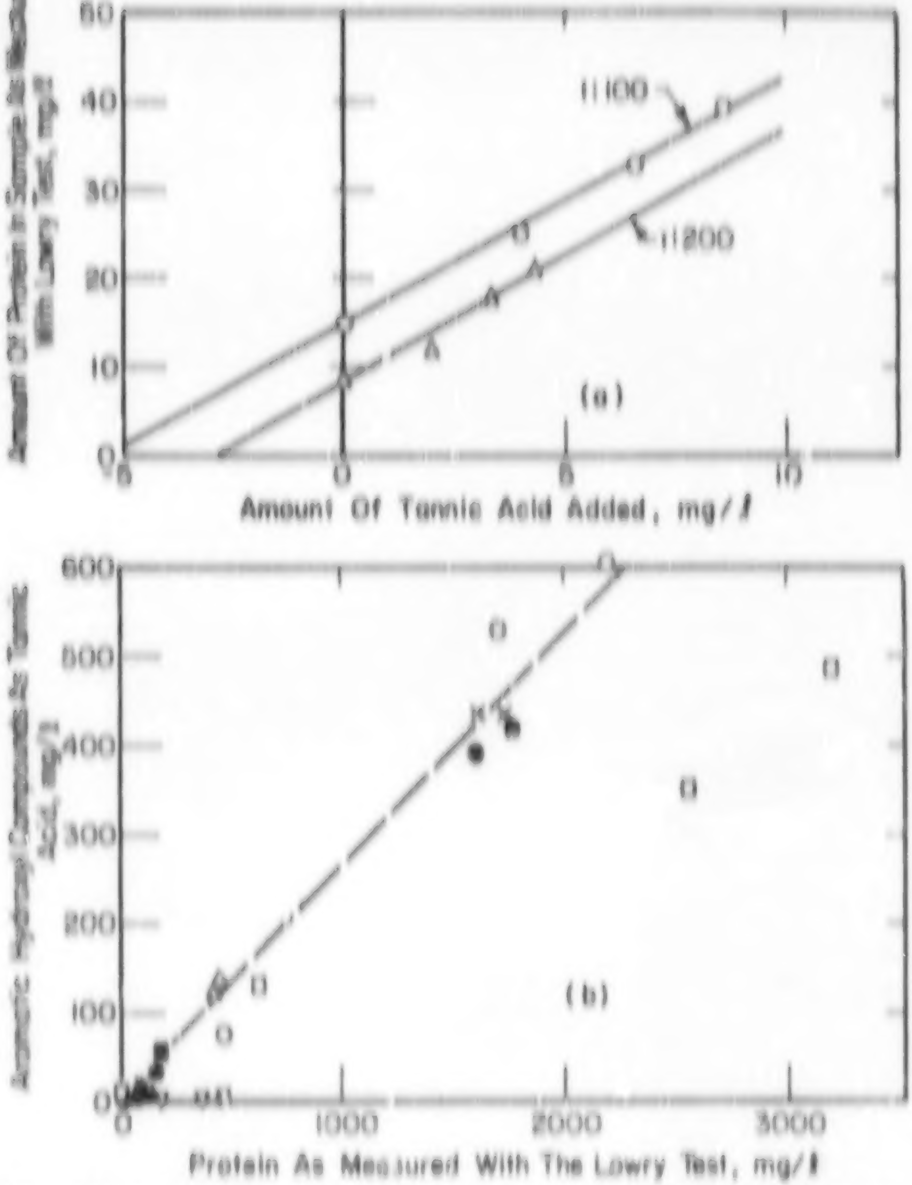

Figure 43. Effect of Standard Additions of Tannic Acid in Leachate on the Outcome of the Lowry Test (a) and Relation Between Results of the Aromatic Hydroxyl Test and Lowry Test as Determined on Different Leachate Samples

reaction extensively to obtain a reliable method for the automatic analysis of amino acids. Yemm and Cocking (1955) described the first step in the reaction as the oxidative deamination of amino acid with the formation of ammonia and the reduction of ninhydrin to hydrantin. The ammonia then condenses with the hydrindantin to form a colored complex.

The test has been used in Painter and Viney (1959) to measure free amino acids in sewage. Stevenson and Cheng (1970) used the ninhydrin method after extensive acid hydrolyses to determine amino acids in sediment samples. A sample is hydrolyzed with 6 N HCl. After removal of HCl the solution is made alkaline and ammonia and amine are removed. Sufficient 6 N HCl is then added to dissolve metallic hydroxides after which the amino acids are analyzed according to the method of Yemm and Cocking (1955), with the exception that excess sodium citrate is applied to chelate metals. The standard amino acid, usually leucine, is subjected to an identical digestion step which generally reduces its concentration by 5-10 percent. It was shown that some amino acids, especially tryptophane, are less resistant to digestions than other amino acids.

PROCEDURES OF THE NINHYDRIN METHOD

After several trials the following procedure was selected as the most satisfactory one:

1. Add 5 ml sample to 25 ml of 6 N HCl and reflux for 20 hours.
2. Filter and wash residue.
3. Add 2-3 drops phenolphthalein, titrate to pink color with 6 N NaOH.
4. Boil to remove NH_4^+.
5. Add 6 N HCl to dissolve metal hydroxide and dilute to 100 ml with boiled water.
6. Take a 1-ml aliquot and add 1 ml sodium citrate solution.
7. Add 4 ml of freshly prepared ninhydrin reagent mix as described by Yemm and Cocking (1955).
8. Place in boiling water bath for 3 minutes.

9. Remove from bath and immediately add 10 ml of 47.5 percent ethanol water mixture.
10. Cool to room temperature and read absorbance at 570 m.

RESULTS OF THE NINHYDRIN METHOD
The results of the test with different leachate samples were found to be accurate and reproducible; a duplicate test with the U of I leachate, for example, showed values of 2420 and 2440 mg/l.

The evaluation of the digestion time showed that high readings are even obtained without hydrolysis (Figure 44a) indicating that most proteins are present in leachate as single amino acids. Analysis with an Automated Technicon Amino acid analyzer showed that most amino acids were present as ornithine, lysine and valine confirming the high organic nitrogen values measured in the 500 MW UF permeate. Since these amino acids do not have aromatic hydroxyl groups, they will not register with the Lowry test. A digestion time of 20 hours was found to be suitable (Figure 44).

Comparison of the protein concentration determined with both the Kjeldahl method and the ninhydrin test showed that the results obtained with each test agree well with each other (Figure 44b). The protein value obtained with the Kjeldahl method was calculated by multiplying the value for N as obtained after digestion and distillation by a factor of 6.25 (Bunch et al., 1961).

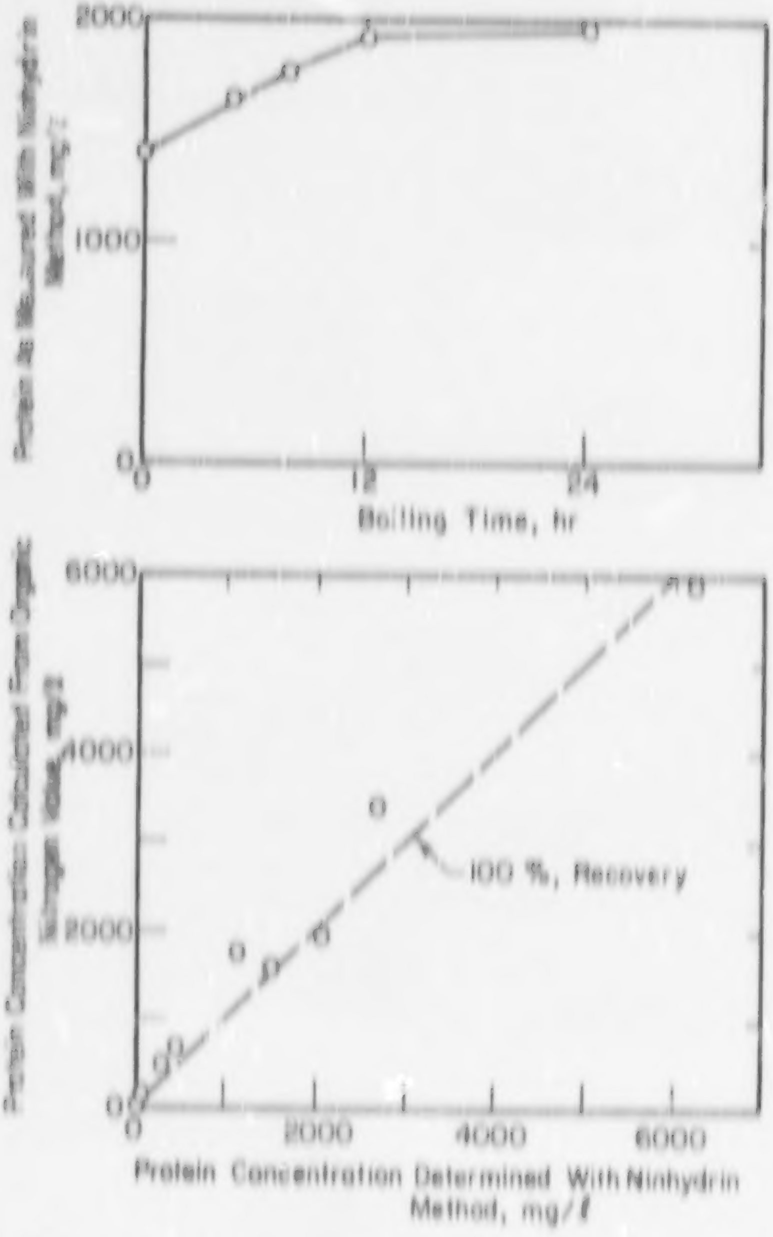

Figure 44. Effect of Digestion Time on Outcome of Ninhydrin Protein Test (a) and Relation Between Protein Concentration as Measured with Kjeldahl Method and Ninhydrin Method Using Digestion

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THE DETERMINATION OF EXTRACTABLE ORGANICS USING HEXANE AND BUTANOL

THE SOLVENT EXTRACTION METHOD

The solvent extraction technique has been used to fractionate organics according to their polarity. Ethanol is for example used to obtain the hymatomelanic acid from the pH 2 precipitated humic acid. Several investigations in the soil science area have used extraction techniques using benzene-methanol, chloroform-methanol, acetyl acetone, hexa methylene, tetramine, dodecylsulfate, urea, formic acid, phenol and acetone to obtain humic materials (Schnitzer and Kahn, 1972). The solvent extraction scheme of Bunch *et al.* (1961) as modified by Rubhun and Manka (1971) was initially used in the present study and mathanol was used to extract the fulvic acids from the solution. Unrealistic high values however were obtained for fulvic acid concentrations. A more in-depth evaluation showed that when the permeate of the 10,000 MW ultrafilter was extracted, both fulvic acids and short-chain free volatile fatty acids were removed from the solution. The latter compounds were detected when the residue of the dried butanol layer was resuspended and GC analysis showed substantial amounts of free volatile fatty acids.

EXTRACTION OF MODEL COMPOUNDS

To study more precisely how many other compounds besides fulvic acid are extracted, a butanol extraction was performed on model compounds that resemble the organics that are expected to be present in leachate such as fulvic acid (tannic acid), carbohydrates (starch), and proteins (bovine albumin). The results in Figure 45 show that the butanol extraction of tannic acid removed most of the compound from the water layer. A comparison of the gravimetric and the colorimetric determination showed that the latter method resulted in lower values as compared with the former method. The extraction of the bovine albumin is less effective than that of tannic acid, however since it is extracted to a considerable extent it

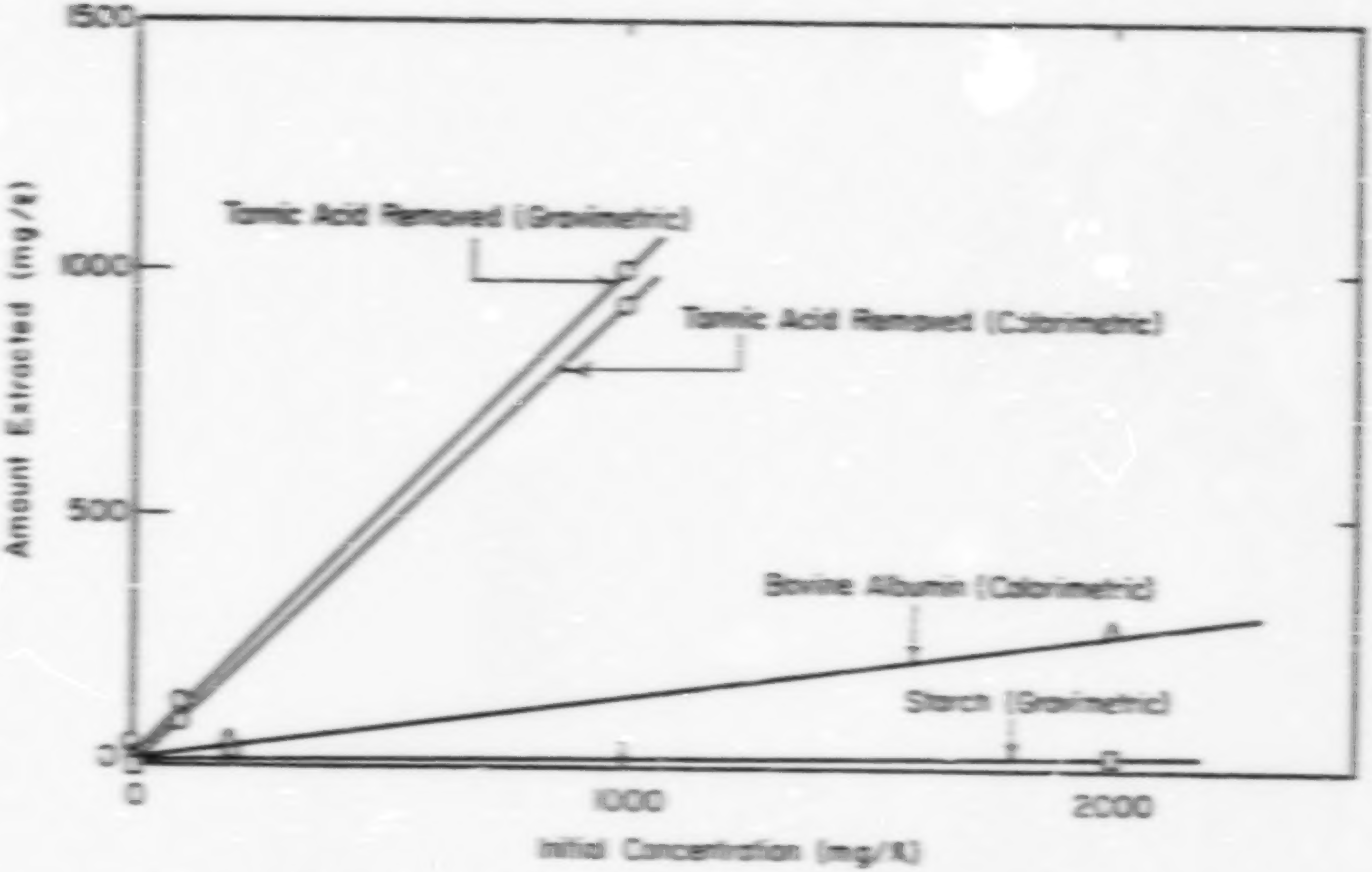

Figure 45. Butanol Extraction of Tannic Acid, Starch and Bovine Albumin

will interfere in the gravimetric determination of the fulvic acid as obtained in the dried butanol residue. The extraction of starch is almost negligible and will not result in any interference.

A second extraction test was conducted with a mixture of free volatile fatty acids as encountered in fresh leachate (formic acid 2000 mg/l, acetic acid 5000 mg/l, propionic acid 1000 mg/l, butyric acid 5000 mg/l, isobutyric acid 1000 mg/l, valeric acid 1000 mg/l, isovaleric acid 1000 mg/l and caproic acid 6000 mg/l). The extraction was performed similarly to that employed in the leachate samples; a 100 ml aliquot is extracted with two 100 ml of hexane. Following the hexane extraction the sample is acidified to pH 2 to obtain a possible precipitate of humic acid, after which the water layer is extracted with butanol. The results of both tests show that large amount of free volatile fatty acid are extracted into the butanol and hexane layer (Figure 46 and 47). After the drying of the hexane and butanol layer at 60°C for 2 days the residues were redissolved in 100 ml water and the TOC was measured. Because of the extensive evaporation of the fatty acids only a fraction was left in the residue as shown in Table 19. The TOC of the redissolved hexane residue was found to be proportional to the initial acid concentration in the water layer while the subsequent butanol extraction removed more fatty acids at the lowest fatty acid concentration. The results also show that the accuracy of the residue determination can vary by more than 50%. For these reasons the extraction method was only applied using the hexane to determine oils or lipids and butanol to detect fulvic acids in the high molecular weight retentate. Application of the extraction in the 500 Mw UF permeate will lead to substantial interferences due to the presence of free volatile fatty acids in leachate.

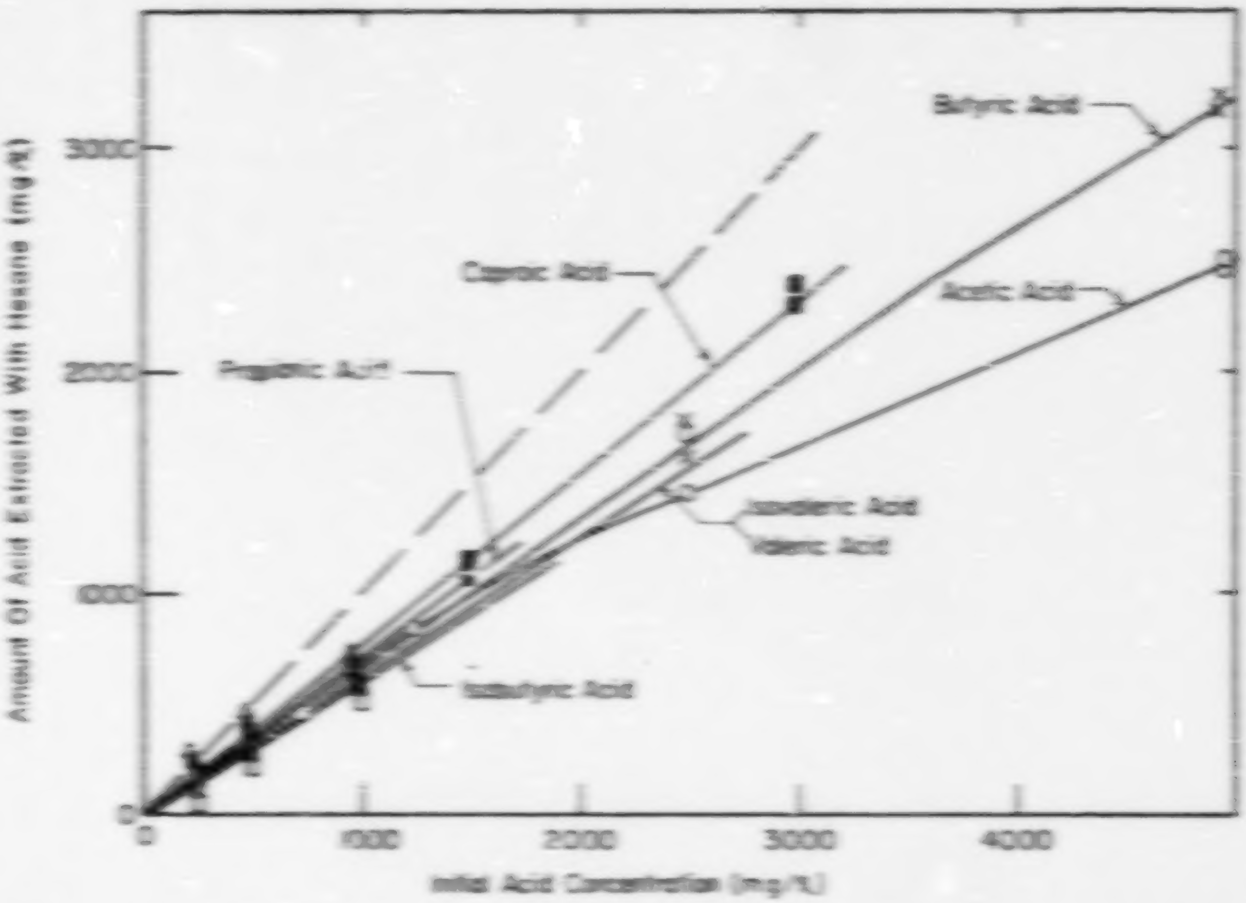

Figure 46. Hexane Extraction of Free Volatile Fatty Acid Mixture

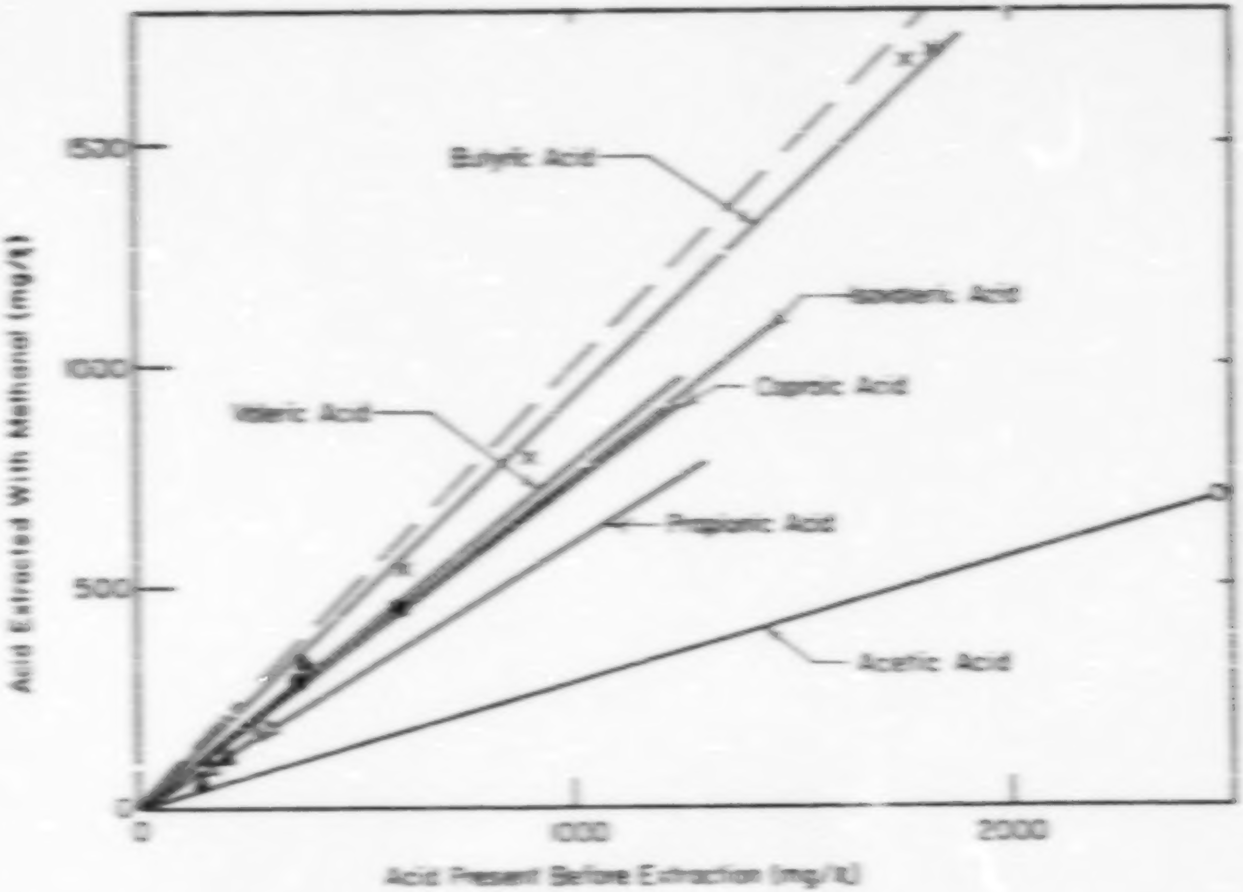

Figure 47. Butanol Extraction of Acidified Free Volatile Fatty Acid Mixture

Table 18

TOC in Redissolved Dried Residue of the Hexane and Butanol
Used to Extract a Free Volatile Fatty Acid
Solutions the Residue Was Redissolved in 100 ml Distilled Water

TOC of Water Layer (mg/l)	TOC of Redissolved Hexane Layer (mg/l)		TOC of Redissolved Butanol Layer (mg/l)	
	Test 1	Test 2	Test 1	Test 2
9318 (initial stock)	167	145	120	120
465 (1:2)	61	98	120	180
2329 (1:4)	1	4	190	160

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